# WEST Search History

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DATE: Thursday, May 10, 2007

| Hide? | Set Name | Query |
|---|---|---|
| | | *DB=PGPB,USPT,EPAB; PLUR=YES; OP=ADJ* |
| ☐ | L65 | l64 and (conjugat$ or link$ or coupl$) |
| ☐ | L64 | L63 not @ay>2000 |
| ☐ | L63 | L62 and antibod$ |
| ☐ | L62 | L61 and l50 |
| ☐ | L61 | IR3 or 5C3 or MC192 |
| ☐ | L60 | L59 and (conjugat$ or link$ or coupl$) |
| ☐ | L59 | L58 and l54 |
| ☐ | L58 | L57 not @ay>2000 |
| ☐ | L57 | antibod$ and L56 |
| ☐ | L56 | L55 and l50 |
| ☐ | L55 | L54.clm. or l54.ab. or l54.ti. |
| ☐ | L54 | (IGF-1R) or (IGF 1R) or (IGFR) or (insulin growth factor type 1) |
| ☐ | L53 | L52 not @ay>2000 |
| ☐ | L52 | L51 and antibod$ |
| ☐ | L51 | L50 AND L49 |
| ☐ | L50 | (cancer$ or tumor$ or neoplas$) |
| ☐ | L49 | l44 and l47 |
| ☐ | L48 | p45 and L47 |
| ☐ | L47 | L46.ab. or l46.clm. or l46.ti. |
| ☐ | L46 | neurotrophin receptor |
| ☐ | L45 | neutrophin receptor |
| ☐ | L44 | p75 |
| ☐ | L43 | L40 and L42 |
| ☐ | L42 | L39 and L41 |
| ☐ | L41 | internal$ |
| ☐ | L40 | dox$ or (taxol or paclitaxel) |
| ☐ | L39 | ("4997913"\|"5084560"\|"5208323"\|"5258453"\|"5869045"\|"6020145"\|"6030997"\|"6140100" |
| ☐ | L38 | L37 and L36 |
| ☐ | L37 | L21 and L29 |
| ☐ | L36 | antibod$ |

- ☐ L35 L34 not @ay>2001
- ☐ L34 L33 and chemotherap$
- ☐ L33 L31 and conjugat$
- ☐ L32 L31 and conjugat?
- ☐ L31 L30 and antibod$
- ☐ L30 L29.ab.
- ☐ L29 p-glycoprotein
- ☐ L28 L27 and antibod$
- ☐ L27 (4062831 or 4097470).pn.
- ☐ L26 L24 and MDR
- ☐ L25 L24 and MRD
- ☐ L24 L23 and antibod$
- ☐ L23 L22 and L21
- ☐ L22 doxorubicin
- ☐ L21 hpma
- ☐ L20 L18 and antibod$
- ☐ L19 L18 and conjugat$
- ☐ L18 L17 or L16
- ☐ L17 guillemard.in.
- ☐ L16 saragovi.in.
- ☐ L15 L14 and L13
- ☐ L14 Singh.in.
- ☐ L13 L12 and L11
- ☐ L12 antibod$.ab.
- ☐ L11 IGF.ab.
- ☐ L10 L9.ti.
- ☐ L9 anti-igf
- ☐ L8 L7 and link$
- ☐ L7 L6 and conjugat$
- ☐ L6 L5 with antibod$
- ☐ L5 IR3
- ☐ L4 MC192
- ☐ L3 L2 and L1
- ☐ L2 MDR
- ☐ L1 ADEPT

END OF SEARCH HISTORY

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| NEWS 1 | | Web Page for STN Seminar Schedule - N. America |
| NEWS 2 | JAN 08 | CHEMLIST enhanced with New Zealand Inventory of Chemicals |
| NEWS 3 | JAN 16 | CA/CAplus Company Name Thesaurus enhanced and reloaded |
| NEWS 4 | JAN 16 | IPC version 2007.01 thesaurus available on STN |
| NEWS 5 | JAN 16 | WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification data |
| NEWS 6 | JAN 22 | CA/CAplus updated with revised CAS roles |
| NEWS 7 | JAN 22 | CA/CAplus enhanced with patent applications from India |
| NEWS 8 | JAN 29 | PHAR reloaded with new search and display fields |
| NEWS 9 | JAN 29 | CAS Registry Number crossover limit increased to 300,000 in multiple databases |
| NEWS 10 | FEB 15 | PATDPASPC enhanced with Drug Approval numbers |
| NEWS 11 | FEB 15 | RUSSIAPAT enhanced with pre-1994 records |
| NEWS 12 | FEB 23 | KOREAPAT enhanced with IPC 8 features and functionality |
| NEWS 13 | FEB 26 | MEDLINE reloaded with enhancements |
| NEWS 14 | FEB 26 | EMBASE enhanced with Clinical Trial Number field |
| NEWS 15 | FEB 26 | TOXCENTER enhanced with reloaded MEDLINE |
| NEWS 16 | FEB 26 | IFICDB/IFIPAT/IFIUDB reloaded with enhancements |
| NEWS 17 | FEB 26 | CAS Registry Number crossover limit increased from 10,000 to 300,000 in multiple databases |
| NEWS 18 | MAR 15 | WPIDS/WPIX enhanced with new FRAGHITSTR display format |
| NEWS 19 | MAR 16 | CASREACT coverage extended |
| NEWS 20 | MAR 20 | MARPAT now updated daily |
| NEWS 21 | MAR 22 | LWPI reloaded |
| NEWS 22 | MAR 30 | RDISCLOSURE reloaded with enhancements |
| NEWS 23 | APR 02 | JICST-EPLUS removed from database clusters and STN |
| NEWS 24 | APR 30 | GENBANK reloaded and enhanced with Genome Project ID field |
| NEWS 25 | APR 30 | CHEMCATS enhanced with 1.2 million new records |
| NEWS 26 | APR 30 | CA/CAplus enhanced with 1870-1889 U.S. patent records |
| NEWS 27 | APR 30 | INPADOC replaced by INPADOCDB on STN |
| NEWS 28 | MAY 01 | New CAS web site launched |
| NEWS 29 | MAY 08 | CA/CAplus Indian patent publication number format defined |

| | |
|---|---|
| NEWS EXPRESS | NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT MACINTOSH VERSION IS V6.0ç(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006. |
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| NEWS LOGIN | Welcome Banner and News Items |
| NEWS IPC8 | For general information regarding STN implementation of IPC 8 |

Enter NEWS followed by the item number or name to see news on that specific topic.

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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007

=> file caplus
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
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FILE LAST UPDATED: 9 May 2007  (20070509/ED)

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http://www.cas.org/infopolicy.html

=> s p75
L1          3555 P75

=> s neurotrophin receptor
            5950 NEUROTROPHIN
            2953 NEUROTROPHINS
            6713 NEUROTROPHIN
                   (NEUROTROPHIN OR NEUROTROPHINS)
          698672 RECEPTOR
          641112 RECEPTORS
          832231 RECEPTOR
                   (RECEPTOR OR RECEPTORS)
L2          1651 NEUROTROPHIN RECEPTOR
                   (NEUROTROPHIN(W)RECEPTOR)

=> s l1 and l2
L3           806 L1 AND L2

=> s cancer or tumor or neoplas
=> s cancer? or tumor? or neoplas?
          330454 CANCER?
          468110 TUMOR?
          491900 NEOPLAS?
L4        775765 CANCER? OR TUMOR? OR NEOPLAS?

=> s l3 adn l4
MISSING OPERATOR L3 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.
```

```
=> s l3 and l4
L5         108 L3 AND L4

=> s antibod?
L6      490570 ANTIBOD?

=> s l6 and l5
L7           6 L6 AND L5

=> d ibib 1-6

L7   ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2002:927183  CAPLUS
DOCUMENT NUMBER:          138:29102
TITLE:                    High affinity ligand for p75
                          neurotrophin receptor
INVENTOR(S):              Hempstead, Barbara L.; Lee, Ramee; Teng, Kenneth K.;
                          Kermani, Pouneh
PATENT ASSIGNEE(S):       Cornell Research Foundation, Inc., USA
SOURCE:                   PCT Int. Appl., 124 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
     WO 2002096356         A2    20021205    WO 2002-US16540         20020524
     WO 2002096356         A3    20060518
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
             LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
             PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
             UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
             CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
             BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG,
             AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
     CA 2447986            A1    20021205    CA 2002-2447986         20020524
     US 2003087804         A1    20030508    US 2002-155886          20020524
     EP 1575477            A2    20050921    EP 2002-729305          20020524
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.:                       US 2001-293823P     P  20010525
                                             US 2001-305510P     P  20010713
                                             WO 2002-US16540     W  20020524
OTHER SOURCE(S):          MARPAT 138:29102

L7   ANSWER 2 OF 6  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2001:863089  CAPLUS
DOCUMENT NUMBER:          136:116439
TITLE:                    Neurotrophins and neurotrophin
                          receptors in human lung cancer
AUTHOR(S):                Ricci, Alberto; Greco, Stefania; Mariotta, Salvatore;
                          Felici, Laura; Bronzetti, Elena; Cavazzana, Andrea;
                          Cardillo, Giuseppe; Amenta, Francesco; Bisetti,
                          Alberto; Barbolini, Giuseppe
CORPORATE SOURCE:         Dipartimento di Scienze Cardiovascolari e
                          Respiratorie, Universita "La Sapienza", Rome, 00151,
                          Italy
SOURCE:                   American Journal of Respiratory Cell and Molecular
                          Biology (2001), 25(4), 439-446
```

CODEN: AJRBEL; ISSN: 1044-1549
PUBLISHER: American Thoracic Society
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:642247 CAPLUS
DOCUMENT NUMBER: 132:149912
TITLE: Expression of p75LNGFR and Trk neurotrophin receptors in normal and neoplastic human prostate
AUTHOR(S): Guate, J. L.; Fernandez, N.; Lanzas, J. M.; Escaf, S.; Vega, J. A.
CORPORATE SOURCE: Servicios de Urologia Hospital San Agustin, Aviles, Spain
SOURCE: BJU International (1999), 84(4), 495-502
CODEN: BJINFO; ISSN: 1464-4096
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:412962 CAPLUS
DOCUMENT NUMBER: 127:106248
TITLE: Immortalization and controlled in vitro differentiation of murine multipotent neural crest stem cells
AUTHOR(S): Rao, Mahendra S.; Anderson, David J.
CORPORATE SOURCE: Division of Biology 216-76, Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA, 91125, USA
SOURCE: Journal of Neurobiology (1997), 32(7), 722-746
CODEN: JNEUBZ; ISSN: 0022-3034
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 69 THERE ARE 69 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:296246 CAPLUS
DOCUMENT NUMBER: 127:15826
TITLE: Differential regulation of two sets of mesonephric tubules by WT-1
AUTHOR(S): Sainio, Kirsi; Hellstedt, Paavo; Kreidberg, Jordan A.; Saxen, Lauri; Sariola, Hannu
CORPORATE SOURCE: Institute of Biotechnology, Program of Developmental Biology, University of Helsinki, Finland
SOURCE: Development (Cambridge, United Kingdom) (1997), 124(7), 1293-1299
CODEN: DEVPED; ISSN: 0950-1991
PUBLISHER: Company of Biologists
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:260381 CAPLUS
DOCUMENT NUMBER: 126:315564

TITLE: Neurotrophins and their receptors in nerve injury and repair
AUTHOR(S): Ebadi, M.; Bashir, R. M.; Heidrick, M. L.; Hamada, F. M.; El Refaey, H.; Hamed, A.; Helal, G.; Baxi, M. D.; Cerutis, D. R.; Lassi, N. K.
CORPORATE SOURCE: Dep. Pharmacology, Univ. Nebraska College Med., Omaha, NE, 68198-6260, USA
SOURCE: Neurochemistry International (1997), 30(4/5), 347-374
CODEN: NEUIDS; ISSN: 0197-0186
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 251 THERE ARE 251 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

```
=> s TRKA or neutrophilic receptor tyrosine kinase
          2288 TRKA
          2821 NEUTROPHILIC
        698672 RECEPTOR
        641112 RECEPTORS
        832231 RECEPTOR
                  (RECEPTOR OR RECEPTORS)
        159381 TYROSINE
          2661 TYROSINES
        159919 TYROSINE
                  (TYROSINE OR TYROSINES)
        289994 KINASE
         55981 KINASES
        299105 KINASE
                  (KINASE OR KINASES)
             0 NEUTROPHILIC RECEPTOR TYROSINE KINASE
                  (NEUTROPHILIC(W)RECEPTOR(W)TYROSINE(W)KINASE)
L8         2288 TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE

=> s TRKA or (neutrophilic receptor tyrosine kinase)
          2288 TRKA
          2821 NEUTROPHILIC
        698672 RECEPTOR
        641112 RECEPTORS
        832231 RECEPTOR
                  (RECEPTOR OR RECEPTORS)
        159381 TYROSINE
          2661 TYROSINES
        159919 TYROSINE
                  (TYROSINE OR TYROSINES)
        289994 KINASE
         55981 KINASES
        299105 KINASE
                  (KINASE OR KINASES)
             0 NEUTROPHILIC RECEPTOR TYROSINE KINASE
                  (NEUTROPHILIC(W)RECEPTOR(W)TYROSINE(W)KINASE)
L9         2288 TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)

=> d his

     (FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

     FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
L1          3555 S P75
L2          1651 S NEUROTROPHIN RECEPTOR
L3           806 S L1 AND L2
L4        775765 S CANCER? OR TUMOR? OR NEOPLAS?
```

```
L5          108 S L3 AND L4
L6       490570 S ANTIBOD?
L7            6 S L6 AND L5
L8         2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9         2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)

=> s l9 and l4
L10         420 L9 AND L4

=> s l10 and l6
L11          95 L10 AND L6

=> s target? and l11
        526093 TARGET?
L12          21 TARGET? AND L11

=> s immunoconjugate or (conjugat? or link? or coupl?)
           1074 IMMUNOCONJUGATE
           2329 IMMUNOCONJUGATES
           2626 IMMUNOCONJUGATE
                  (IMMUNOCONJUGATE OR IMMUNOCONJUGATES)
         235566 CONJUGAT?
         493832 LINK?
         823725 COUPL?
L13     1486130 IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)

=> s l13 and l11
L14          26 L13 AND L11

=> s l14 and l12
L15           8 L14 AND L12

=> s l15 not py>2002
       5237136 PY>2002
L16           1 L15 NOT PY>2002

=> d ibib

L16  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2001:125543  CAPLUS
DOCUMENT NUMBER:          134:348031
TITLE:                    Taxane-antibody conjugates afford
                          potent cytotoxicity, enhanced solubility, and
                          tumor target selectivity
AUTHOR(S):                Guillemard, Veronique; Saragovi, H. Uri
CORPORATE SOURCE:         Departments of Pharmacology and Therapeutics, McGill
                          University, Montreal, QC, H3G 1Y6, Can.
SOURCE:                   Cancer Research (2001), 61(2), 694-699
                          CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER:                American Association for Cancer Research
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
REFERENCE COUNT:          24    THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> s l14 not py>2001
       6209673 PY>2001
L17           3 L14 NOT PY>2001

=> d ibib 1-3

L17  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2001:125543  CAPLUS
```

DOCUMENT NUMBER: 134:348031
TITLE: Taxane-antibody conjugates afford potent cytotoxicity, enhanced solubility, and tumor target selectivity
AUTHOR(S): Guillemard, Veronique; Saragovi, H. Uri
CORPORATE SOURCE: Departments of Pharmacology and Therapeutics, McGill University, Montreal, QC, H3G 1Y6, Can.
SOURCE: Cancer Research (2001), 61(2), 694-699
CODEN: CNREA8; ISSN: 0008-5472
PUBLISHER: American Association for Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:872516 CAPLUS
DOCUMENT NUMBER: 124:5972
TITLE: Trk A gene expression in neuroblastoma: The clinical significance of an immunohistochemical study
AUTHOR(S): Tanaka, Takeo; Hiyama, Eiso; Sugimoto, Tohru; Sawada, Tadashi; Tanabe, Masahiro; Ida, Noriaki
CORPORATE SOURCE: Department Pediatrics, National Kure Hospital, Kure, 737, Japan
SOURCE: Cancer (New York) (1995), 76(6), 1086-95
CODEN: CANCAR; ISSN: 0008-543X
PUBLISHER: Lippincott-Raven
DOCUMENT TYPE: Journal
LANGUAGE: English

L17 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:579637 CAPLUS
DOCUMENT NUMBER: 122:307343
TITLE: Nerve growth factor as a mitogen for a pancreatic carcinoid cell line
AUTHOR(S): Bold, Richard J.; Ishizuka, Jin; Rajaraman, Srinivasan; Perez-Polo, J. Regino; Townsend, Courtney M., Jr.; Thompson, James C.
CORPORATE SOURCE: Dep. Surgery, Univ. Texas Medical Branch, Galveston, TX, USA
SOURCE: Journal of Neurochemistry (1995), 64(6), 2622-8
CODEN: JONRA9; ISSN: 0022-3042
PUBLISHER: Lippincott-Raven
DOCUMENT TYPE: Journal
LANGUAGE: English

```
=> s insulin () growth factor receptor
        202363 INSULIN
          5286 INSULINS
        202444 INSULIN
                 (INSULIN OR INSULINS)
       1344531 GROWTH
          4492 GROWTHS
       1346816 GROWTH
                 (GROWTH OR GROWTHS)
       1036648 FACTOR
        936905 FACTORS
       1634156 FACTOR
                 (FACTOR OR FACTORS)
        698672 RECEPTOR
        641112 RECEPTORS
        832231 RECEPTOR
                 (RECEPTOR OR RECEPTORS)
```

```
         41650 GROWTH FACTOR RECEPTOR
                 (GROWTH(W)FACTOR(W)RECEPTOR)
L18        108 INSULIN (W) GROWTH FACTOR RECEPTOR

=> s (IGF-1R) or (IGF1R) or (IGF 1R)
         28084 IGF
          2542 IGFS
         28212 IGF
                 (IGF OR IGFS)
         14865 1R
           496 IGF-1R
                 (IGF(W)1R)
           380 IGF1R
         28084 IGF
          2542 IGFS
         28212 IGF
                 (IGF OR IGFS)
         14865 1R
           496 IGF 1R
                 (IGF(W)1R)
L19        819 (IGF-1R) OR (IGF1R) OR (IGF 1R)

=> s l19 or l18
L20        914 L19 OR L18

=> d his

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     FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
L1        3555 S P75
L2        1651 S NEUROTROPHIN RECEPTOR
L3         806 S L1 AND L2
L4      775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5         108 S L3 AND L4
L6      490570 S ANTIBOD?
L7           6 S L6 AND L5
L8        2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9        2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10        420 S L9 AND L4
L11         95 S L10 AND L6
L12         21 S TARGET? AND L11
L13    1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14         26 S L13 AND L11
L15          8 S L14 AND L12
L16          1 S L15 NOT PY>2002
L17          3 S L14 NOT PY>2001
L18        108 S INSULIN () GROWTH FACTOR RECEPTOR
L19        819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20        914 S L19 OR L18

=> s type 1 and l18
       1790611 TYPE
        611407 TYPES
       2263312 TYPE
                 (TYPE OR TYPES)
       9125368 1
         73341 TYPE 1
                 (TYPE(W)1)
L21          7 TYPE 1 AND L18

=> s l19 and l4
L22        417 L19 AND L4
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```
=> s l22 and l6
L23          83 L22 AND L6

=> s l23 and l13
L24          18 L23 AND L13

=> s l24 not py>2001
       6209673 PY>2001
L25           2 L24 NOT PY>2001

=> d ibib 1-2

L25  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         1999:336320  CAPLUS
DOCUMENT NUMBER:          131:128377
TITLE:                    Expression of the insulin-like growth factor 1
                          receptor (IGF-1R) in breast
                          cancer cells: evidence for a regulatory role
                          of dolichyl phosphate in the transition from an
                          intracellular to an extracellular IGF-1 pathway
AUTHOR(S):                Dricu, Anica; Kanter, Lena; Wang, Min; Nilsson,
                          Gunnar; Hjertman, Magnus; Wejde, Johan; Larsson, Olle
CORPORATE SOURCE:         Cellular and Molecular Tumor Pathology, CCK, R8:04,
                          Karolinska Hospital, Stockholm, S-17176, Swed.
SOURCE:                   Glycobiology (1999), 9(6), 571-579
                          CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER:                Oxford University Press
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
REFERENCE COUNT:          34    THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L25  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         1998:798140  CAPLUS
DOCUMENT NUMBER:          130:163531
TITLE:                    Expression of the insulin-like growth factor-1
                          receptor and its anti-apoptotic effect in malignant
                          melanoma: a potential therapeutic target
AUTHOR(S):                Kanter-Lewensohn, L.; Dricu, A.; Wang, M.; Wejde, J.;
                          Kiessling, R.; Larsson, O.
CORPORATE SOURCE:         Cellular and Molecular Tumor Pathology, CCK, R8:04,
                          Karolinska Hospital, Stockholm, 171 76, Swed.
SOURCE:                   Melanoma Research (1998), 8(5), 389-397
                          CODEN: MREEEH; ISSN: 0960-8931
PUBLISHER:                Lippincott-Raven Publishers
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LANGUAGE:                 English
REFERENCE COUNT:          20    THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> d kwic 1-2

L25  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
TI   Expression of the insulin-like growth factor 1 receptor (IGF-
     1R) in breast cancer cells: evidence for a regulatory
     role of dolichyl phosphate in the transition from an intracellular to an
     extracellular IGF-1 pathway
AB   In this study we provide evidence that the low expression of IGF
     -1R at the cell surface of estrogen-independent breast
     cancer cells is due to a low rate of de novo synthesis of dolichyl
     phosphate.  The analyses were performed on the estrogen receptor-neg.
     breast cancer cell line MDA231 and, in comparison, the melanoma
     cell line SK-MEL-2, which expresses a high number of plasma membrane-bound
```

IGF-1R. Whereas the MDA231 cells had little or no surface expression of IGF-1R, they expressed functional (i.e., ligand-binding) intracellular receptors. By measuring the incorporation of [3H]mevalonate into dolichyl phosphate, we could demonstrate that the rate of dolichyl phosphate synthesis was considerably lower in MDA231 cells than in SK-MEL-2 cells. Furthermore, N-linked glycosylation of the α-subunit of IGF-1R was 8-fold higher in the melanoma cells. Following addition of dolichyl phosphate to MDA231 cells, N-linked glycosylation of IGF-1R was drastically increased, which in turn was correlated to a substantial translocation of IGF-1R to the plasma membrane, as assayed by IGF-1 binding anal. and by Western blotting of plasma membrane proteins. The dolichyl. . . phosphate-stimulated receptors were proven to be biochem. active since they exhibited autophosphorylation. Under normal conditions MDA231 cells, expressing very few IGF-1R at the cell surface, were not growth-arrested by an antibody (αIR-3) blocking the binding of IGF-1 to IGF-1R. However, after treatment with dolichyl phosphate, leading to a high cell surface expression of IGF-1R, αIR-3 efficiently blocked MDA231 cell growth. Taken together with the fact that the breast cancer cells produce IGF-1 and exhibit intracellular binding, our data suggest that the level of de novo-synthesized dolichyl phosphate may be. . .

ST IGF1 receptor breast cancer proliferation dolichyl phosphate

IT Phosphorylation, biological
(autophosphorylation; insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT Gene
(expression; insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT Cell membrane
Cell proliferation
Glycosylation
(insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT Insulin-like growth factor I receptors
RL: ADV (Adverse effect, including toxicity); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT Mammary gland
(neoplasm; insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT 67763-96-6, IGF-1
RL: ADV (Adverse effect, including toxicity); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

IT 12698-55-4, Dolichyl phosphate
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study);

FORM (Formation, nonpreparative); OCCU (Occurrence); PROC (Process)
(insulin-like growth factor 1 receptor expression mediation by de novo synthesis of dolichyl phosphate in human estrogen-independent breast cancer)

L25 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2007 ACS on STN
AB The insulin-like growth factor-1 receptor (IGF-1R) and its possible protective effect on apoptotic cell death in malignant melanoma was analyzed in four com. melanoma cell lines. Inhibition of N-linked glycosylation by tunicamycin, which has previously been shown to block the translocation of IGF-1R to the cell surface, blocked cell growth and/or induced cell death in these cell lines. Treatment with αIR-3, an antibody blocking the binding domain of IGF-1R, also resulted in growth arrest and/or apoptosis. We also analyzed lymph node metastases of malignant melanoma by Western blotting and immunohistochem. All these cases were shown to express IGF-1R at the cell surface. In three cases of lymph node metastases we had access to both tumor specimens and cultured cells. One of these exhibited a substantially higher expression of IGF-1R than the two other cases. The corresponding cell lines showed growth arrest and apoptosis following treatment with αIR-3. However, the two cell lines with low expression of IGF-1R were more sensitive in this respect. Furthermore, we demonstrated an inverse correlation between IGF-1R expression and the frequency of apoptotic cells in the tumor specimens. Our data suggest that IGF-1R is crucial for the viability of malignant melanoma cells in vitro as well as in vivo.
IT Lymph node
(neoplasm, metastasis, melanoma metastasis to; IGF-I receptor expression and its anti-apoptotic effect in malignant melanoma)

=> file reg

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L26           0 5C3/CN

=> s MC192/cn
L27           0 MC192/CN

=> file pctfull
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FILE COVERS 1978 TO DATE

>>> IMAGES ARE AVAILABLE ONLINE AND FOR EMAIL-PRINTS <<<

=> s p75
L28        1765 P75

=> s neurotrophin receptor
           1650 NEUROTROPHIN
            983 NEUROTROPHINS
           2076 NEUROTROPHIN
                  (NEUROTROPHIN OR NEUROTROPHINS)
          89919 RECEPTOR
          66783 RECEPTORS
          99939 RECEPTOR
                  (RECEPTOR OR RECEPTORS)
L29         407 NEUROTROPHIN RECEPTOR
                  (NEUROTROPHIN(W)RECEPTOR)

=> s l28 and l29
L30         208 L28 AND L29

=> s cancer? or tumor? or neoplas?
          88115 CANCER?
          73158 TUMOR?
          25581 NEOPLAS?
L31      109389 CANCER? OR TUMOR? OR NEOPLAS?

=> s antibod?
L32       97772 ANTIBOD?

=> s l32 and l31
L33       63932 L32 AND L31

=> s l29 and l33
L34         322 L29 AND L33

=> s immunoconjugate or (conjugat? or link? or coupl?)
           1229 IMMUNOCONJUGATE
           1863 IMMUNOCONJUGATES
           2279 IMMUNOCONJUGATE
```

```
                (IMMUNOCONJUGATE OR IMMUNOCONJUGATES)
         84377 CONJUGAT?
        336456 LINK?
        374001 COUPL?
L35     568977 IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)

=> s l35 and l34
L36        316 L35 AND L34

=> s l36 not py>2001
        641685 PY>2001
L37        105 L36 NOT PY>2001

=> s l36 not py>2000
        740172 PY>2000
L38         79 L36 NOT PY>2000

=> d ibib kwic

L38    ANSWER 1 OF 79    PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:       2001058954 PCTFULL
       no bibliographic data available - please use FPI for PI information
DESIGNATED STATES

DETD   BACKGROUND OF THE INVENTION
       The tumor necrosis factor receptor (TNF-R) family members play
       key roles in
       the regulation of cell survival and death decisions (Baker and Reddy,.
       . .
 . . .
       transmembrane proteins, this family includes a
       soluble secreted protein, e.g. OPG (Emery et al., 1998, J ofBiol Chem,
       273:14363-
       14367), and a gpi-linked protein, DcR1 (Degli-Esposti, 1999,
       JqfLeukocyte Biology,
       65:535-542).
 . . .
       to a nucleic acid molecule encoding a TRADEcc polypeptide
       or TRADEP polypeptide or portion thereof. In another embodiment, the
       agent is an
         antibody that recognizes a TRADE family member polypeptide. In
       still another
       1 5 embodiment, the activity is selected from the group consisting.  .
       .
 . . .
       antisense to a nucleic acid molecule encoding a TRADE family polypeptide
       or
       portion thereof. In one embodiment, the agent is an antibody
       that recognizes a TRADE
       family polypeptide.

       In one embodiment, the disorder is a proliferative disease or disorder
       selected
       from the group consisting of- inflammation and neoplasia. In
       one embodiment, the
         neoplasia is a carcinoma. In one embodiment, the
       neoplasia is present in lung or prostate
       tissue. In one embodiment, the neoplasia is an adenocareinoma
       In another aspect, the invention pertains to a method for treating a
       subject having
       1 5 a carcinoma or.  .  .
 . . .
       pathway refers to any one of the
       signaling pathways known in the art which involve activation or
```

deactivation of the
transcriptionfactorNFkB,andwhichareatleastpartiallymediatedbytheNFkBfact
or
(Karin, 1998, Cancer Jftom Scientific American, 4:92-99;
Wallach et al, 1999, Ann Rev
- 12 -
of1mmunology, 17:331-367). Generally, such NFkB signaling pathway are
responsive. . .
. .
any one of the
signaling pathways known in the art which involve the Jun amino terminal
kinase (JNK)
1 0 (Karin, 1998, Cancer Jftom Scientific American, 4:92-99;
Wallach et al, 1999, Ann Rev
of1mmunology, 17:331-367). This kinase is generally responsive to a
number of
extracellular. . .
. .
techniques, or substantially
free of chemical precursors or other chemicals when chemically
synthesized. An
isolated TRADE nucleic acid molecule may, however, be linked
to other micleotide
sequences that do not normally flank the TRADE sequences in genomic DNA
(e.g., the
TRADE nucleotide sequences may be linked to vector sequences).
In certain preferred
embodiments, an isolated nucleic acid molecule, such as a cDNA molecule,
also may
be free of. . .

- 15 -
As used herein, the term neoplasia refers to a proliferative
disease or disorder
resulting from uncontrolled or abberant cell division. The term
neoplasia includes
malignant and non-malignant disorders. As used herein, the term
adenocarcinoma
refers generally to cancers of glandular. epithelial cells and
carcinoma refers to
malignant epithelial tumors.
. .
herein, the term vector refers to a nucleic acid molecule capable of
transporting another nucleic acid to which it has been linked.
One type of vector is a
plasmid, which refers to a circular double stranded DNA loop into which
additional
DNA segments may. . . replicated
along with the host genome. Moreover, certain vectors are capable of
directing the
expression of genes to which they are operatively linked. Such
vectors are referred to
herein as recombinant expression vectors or simply expression vectors.
In general,
expression vectors of utility in recombinant. . .
. .
a location or locations in the genome that differs from that in which it
occurs
in nature or which is operatively linked to DNA to which it is
not normally linked in
nature (i.e., a gene that has been operatively linked to a
heterologous promoter).

As used herein, the term antibody is intended to include

immunoglobulin
molecules and immunologically active portions of immunoglobulin
molecules, i.e.,
molecules that contain an antigen binding site which binds (immunoreacts
with) an
antigen, such as Fab and F(ab')2 fragments, single chain
antibodies, intracellular
antibodies, scFv, Fd, or other fragments. Preferably,
antibodies of the invention bind
specifically or substantially specifically to TRADE molecules (i.e.,
have little to no cross
reactivity with non-TRADE molecules). The terms monoclonal
antibodies and
monoclonal antibody composition, as used herein, refer to a
population of antibody
molecules that contain only one species of an antigen binding site
capable of
immunoreacting with a particular epitope of an antigen, whereas the term
polyclonal
antibodies and polyclonal antibody composition refer
to a population of antibody
molecules that contain multiple species of antigen binding sites capable
of interacting
- 18 -
with a particular antigen. A monoclonal antibody compositions
thus typically display a
single binding affinity for a particular antigen with which it
immunoreacts.

cells, e.g., excessive or unwanted proliferation
of cells or deficient proliferation of cells. In one embodiment, TRADE
associated
disorders include such as neoplasia or inflammation. Examples
of TRADE associated
1 0 disorders include: disorders involving aberrant or unwanted
proliferation of cells, e.g.,
inflammation, neoplasia, apoptosis, or necrosis. Preferably,
the cells undergoing
unwanted proliferation in a TRADE-associated disorder are epithelial
cells, e.g., of the
lung, liver, brain, intestine, or prostate. Further examples of TRADE
associated
disorders include carcinomas, adenocarcinomas, and other
neoplasias. TRADE-
associated disorders may also include disorders that have been
linked generally to
aberrant TNF receptor activity or fimction, including Crohn's Disease
(Baert and
Rutgeerts, 1999, Int J Colorectal Dis, 14:47-5 1) and.

IL Methods of Use
The nucleic acid molecules, proteins, protein homologues, and
antibodies
described herein can be used in one or more of the following methods: a)
methods of
modulating proliferation of a cell, b).

TRADE protein
or production of TRADE protein forms which have decreased or aberrant
activity
compared to TRADE wild type protein. Moreover, anti-TRADE
antibodies can be used
to detect and isolate TRADE proteins, regulate the bioavailability of
TRADE proteins,

5 and modulate TRADE activity e.g., modulate. . . liver, the brain, the prostate, the lung, or the intestine. In one embodiment, the detection method is performed to determine whether a neoplastic condition exists, e.g., a carcinoma or an adenocarcinoma. In one embodiment of the invention, the subject methods are used (e.g., to modulate. . .

Exemplary inhibitory agents include antisense TRADE nucleic acid molecules (e.g., to inhibit translation of TRADE mRNA), intracellular anti- TRADE antibodies (e.g., to inhibit the activity of TRADE protein), and dominant negative mutants of the TRADE protein. Other inhibitory agents that can be. . .

For stimulatory or inhibitory agents that comprise nucleic acids (including recombinant expression vectors encoding TRADE protein, antisense RNA, intracellular antibodies or dominant negative inhibitors), the agents can be introduced into cells of the subject using methods known in the art for. . .

. . .

5 5,166,320). Binding of the DNA-ligand complex to the receptor facilitates uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which naturally disrupt endosomes, thereby releasing material into the cytoplasm can be used to avoid degradation of the complex. . .

. . .

be detected by an appropriate assay, for example by immunological detection of a produced protein,



such as with a specific antibody, or by a functional assay to detect a functional activity of the gene product.

. . .

apoptosis. In a preferred embodiment, TRADE is modulated to enhance apoptosis of a epithelial cell, such as to promote the apoptosis in cancer cells, e.g., in the lung, liver, brain, intestine or prostate.

. . .

as a nucleic acid or a protein, a naturally-occurring target molecule of a TRADE protein (e.g., a TRADE binding protein), a TRADE antibody, a TRADE agonist or antagonist, a peptidomimetic of a TRADE agonist or antagonist, or other small molecule. In one embodiment, the. . . embodiment, the agent inhibits one or more TRADE activities. Examples of such inhibitory agents include, e.g., antisense TRADE nucleic acid molecules, anti-TRADE antibodies, and TRADE inhibitors. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in. . .

. . .

will be desirable are in the treatment of TRADE-associated disorders, including disorders

involving
aberrant or unwanted proliferation of cells, e.g., inflammation or cancer. Preferably, the
I 0 cells undergoing unwanted proliferation are epithelial cells, e.g., of the lung or prostate.

Further examples of TRADE associated disorders include carcinomas, - adenocarcinomas, and other neoplasias. TRADE disorders may also include disorders
that have been linked generally to aberrant TNF receptor activity or fimction, including
Crohn's Disease (Baert and Rutgeerts, 1999, Int J Colorectal Dis, 14:47-5 1). . .
. .
acid molecules, comprising at least a
first nucleotide sequence encoding a full-length TRADE protein, polypeptide or peptide
having a TRADE activity operatively linked to a second nucleotide sequence encoding a
non- TRADE protein, polypeptide or peptide, can be prepared by standard recombinant
1 5 DNA. . .
. .
be modified such that they specifically bind to
1 5 receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense
nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or
antigens. The antisense nucleic acid molecules can also be delivered to cells using the
vectors. . .
. .
polymerases), to interact witlithe DNA portion while the PNA portion would
provide high binding affinity and specificity. PNA-DNA chimeras can be linked using
linkers of appropriate lengths selected in terms of base stacking, number of bonds
between the nucleobases, and orientation (Hyrup B., 1996, supra).. . .
Res. 24 (17): 3 3 57 For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and
modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine
phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. et
al., 1989, Nucleic Acid Res. 17: 5973-88). PNA monomers are then coupled in a
stepwise manner to produce a chimeric molecule with a TPNA segment and a YDNA
segment (Finn P.J. et al., 1996,. . .
. .
et al., 1988, Bio-Techniques 6:958-976) or
intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the
oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization
I 0 triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

B. Isolated TRADE Proteins, Fragments Thereof, andAnti-TRADE Antibodies
Isolated TRADE proteins, and biologically active portions thereof can also be

used as modulating agents, as well as polypeptide fragments suitable for use as
immunogens to raise anti-TRADE antibodies. In one embodiment, native TRADE
proteins can be isolated from cells or tissue sources by an appropriate purification
scheme using standard protein. . .

. . .
also provides TRADE chimeric or fusion proteins. As used
herein, a TRADE chimeric protein or fasion protein comprises a TRADE
polypeptide operatively linked to a non- TRADE polypeptide. An TRADE
- 49 -
polypeptide refers to a polypeptide having an arnino acid sequence corresponding to
TRADE. . .

Within the fusion protein, the term operatively linked is intended to indicate that the
I 0 TRADE polypeptide and the non-TRADE polypeptide are fused in-frame to each other.

. . .
HA epitope tag). A
TRADE encoding micleic acid can be cloned into such an expression vector such that
the fusion moiety is linked in-frame to the TRADE protein.

. . .
for the treatment of
disorders, e.g., as soluble antagonists of the TRADE ligand. Disorders that would
benefit from such treatment include, e.g. cancer or Alzheimer's disease. Such Fc fusion
proteins can be used as soluble antagonists of the TRADE ligand. Moreover, the
TRADE-fusion proteins of the invention can be used as immunogens to produce anti-
TRADE antibodies in a subject.

. . .
a GST polypeptide). A TRADE-
encoding nucleic acid can be cloned into such an expression vector such that the fusion
moiety is linked in-frame to the TRADE protein.

. . .
paradigm
polypeptide (i.e., a polypeptide that has a biological or pharmacological activity), such
as human TRADE, but have one or more peptide linkages optionally replaced by a
linkage selected from the group consisting of- -CH2NH-, -CH2S-, -CH2-CH2-, -
CH=CH- (cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods known
in. . . (-C(OH)CH2-); and Hruby, V. J., 1982, Life Sci 31:189-199 (-CH2-S-); each of which is incorporated herein by reference. A
particularly preferred non-peptide linkage is -CH2NH-. Such peptide minietics may have
significant advantages over polypeptide embodiments, including, for example: more
economical production, greater chemical stability, enhanced. . .

. . .
antagonists of a TRADE/TRADE binding protein interaction. Peptides
can be produced as modified peptides, with nonpeptide moieties attached by covalent

I 0 linkage to the N-tenninus and/or C-terminus. In certain preferred embodiments, either
the carboxy-terminus or the amino-tenninus, or both, are chemically modified. The
most. . .

An isolated TRADE protein, or a portion or fragment thereof, can also be used
as an immunogen to generate antibodies that bind TRADE using standard techniques for
polyclonal and monoclonal antibody preparation. A full-length TRADE protein can be
used or, alternatively, the invention provides antigenic peptide fragments of TRADE for
use as immunogens.. . . The antigenic peptide of TRADE comprises at least 8 amino acid
residues and encompasses an epitope of TRADE such that an antibody raised against the
peptide foniis a specific. immune complex with TRADE. Preferably, the antigenic
peptidecomprisesatleastlOaminoacidresidues,morepreferablyatleastl5aminoacid
- 57 -
residues, even more preferably at least. . .
. . .
or an amino acid sequence of another TRADE family polypeptide and encompasses an epitope of a TRADE polypeptide such that an antibody raised
against the peptide forms an immune complex with a TRADE molecule. Preferred
epitopes encompassed by the antigenic peptide are regions of TRADE that are located on
1 0 the surface of the protein, e.g., hydrophilic regions. In one embodiment, an antibody
binds substantially specifically to a TRADE molecule. In another embodiment, an
antibody binds specifically to a TRADE polypeptide.

A TRADE immunogen typically is used to prepare antibodies by immunizing a
suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the inuminogen. An
appropriate immunogenic preparation can contain, for. . . or incomplete
adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with
an immunogenic TRADE preparation induces a polyclonal anti- TRADE antibody
response.

- 58 -
Accordingly, another aspect of the invention pertains to the use of anti- TRADE
family polypeptide antibodies. Such antibodies can be used as agonists and/or
antagonists of TRADE family polypeptides. In a prefered embodiment antibodies
specifically recognize TRADEa or P and not another TRADE family polypeptide.

Polyclonal anti-TRADE antibodies can be prepared as described above by immunizing a
suitable subject with a TRADE immunogen. The anti-TRADE antibody titer in the

immunized subject can be monitored over time by standard techniques, such as with an
enzyme linked immunosorbent assay (ELISA) using immobilized a TRADE
polypeptide. If desired, the antibody molecules directed against a TRADE polypeptide
I 0 can be isolated from the mammal (e.g., from the blood) and further purified. . . such as protein A chromatography to obtain the IgG fraction. At an
appropriate time after immunization, e.g., when the anti- TRADE antibody titers are
highest, antibody-producing cells can be obtained from the subject and used to prepare
monoclonal antibodies by standard techniques, such as the hybridoma technique
originally described by Kohler and Milstein (Kohler and Milstein, 1975, Nature
256:495-497) (see also,. . . aL,l 980,
JBiol Chem 255:4980-83; Yeh et al., 1976, Prod.Naff Acad. Sci USA 76:2927-3 1; and
Yeh et al., 1982, Int. J. Cancer 29:269-75), the more recent human B cell hybridorna
technique (Kozbor et al., 1983, Immunol Today 4:72), the EBV-hybridoma technique
(Cole et al., 1985, Monoclonal Antibodies and Cancer
Therapy, Alan R. Liss, Inc., pp.

77-96) or triorna techniques. The technology for producing monoclonal antibody
hybridomas is well known (see generally R. H. Kenneth, in Monoclonal Antibodies: A
New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York
(1980); E. A. Lerner, 1981, Yale J Biol. Med,. . . as described above, and the culture supernatants of the resulting hybridoma
cells are screened to identify a hybridoma producing a monoclonal antibody that binds
specifically to a TRADE polypeptide.

. . .
known protocols used for fusing lymphocytes and
immortalized cell lines can be applied for the purpose of generating an anti- TRADE
monoclonal antibody (see, e.g., G. Galfre et al., 1977, Nature
266:55052; Gefter et al.

Somatic Cell Genet., cited supra; Lerner, Yale J Biol. Med, cited supra; Kenneth,
Monoclonal Antibodies, cited supra). Moreover, the ordinary skilled worker will
appreciate that there are many variations of such methods which also would be. . .

. . .
unfused and unproductively
fused myeloma cells (unfused splenocytes die after several days because they are not
transformed). Hybridoma cells producing a monoclonal antibody of the invention are
detected by screening the hybridorna culture supernatants for antibodies that bind a
TRADE molecule, e.g., using a standard ELISA assay.

As an alternative to preparing monoclonal antibody-secreting hybridomas, a
monoclonal anti-TRADE antibody can be identified and isolated

by screening a
recombinant combinatorial immunoglobulin library (e.g., an
antibody phage display
library) with a TRADE to thereby isolate immunoglobulin library members
that bind a
TRADE polypeptide. Kits for generating and screening phage display
libraries are
commercially available (e.g., the Pharmacia Recombinant Phage
Antibody System,
Catalog No. 27 Ol.- and the Stratagene Sur)Z4PTM Phage Display Kit,
Catalog
No. 240612). Additionally, examples of methods and reagents particularly
amenable for
use in generating and screening antibody display library can
be found in, for example,
- 60 -
Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International. .
.
. . .
Barbas et al., 1991, Proc. Natl. Acad Sci USA 88:7978-7982; and
McCafferty et
al., 1990, Nature 348:552
Additionally, recombinant anti- TRADE antibodies, such as
chimeric and
humanized monoclonal antibodies, comprising both human and
non-human portions,
which can be made using standard recombinant DNA techniques, are within
the scope of
the invention. Such chimeric and humanized monoclonal antibodies
can be produced by
recombinant DNA techniques known in the art, for example using methods
described in
Robinson et al. International Patent. . .
. . .
USA 84:214-218; Nishimura et
al., 1987, Canc. Res. 47:999-1005; Wood et al., 1985, Nature
314:446-449; and Shaw et
al., 1988, J Natl Cancer Inst. 80:1553-1559); Morrison, S. L.,
1985, Science 229:1202-
1207; Oi et al., 1986, BioTechniques 4:214; Winter U.S. Patent
5,225,539; Jones et al.,
1986, Nature 321:552-525; Verhoeyan et al., 1988, Science 239:1534; and
Beidler et al.,
1988, J Iininunol. 141:4053
-
In addition, humanized antibodies can be made according to
standard protocols
such as those disclosed in US patent 5,565,332. In another embodiment,
antibody chains
or specific binding pair members can be produced by recombination
between vectors
comprising nucleic acid molecules encoding a fusion of a. . .

An anti- TRADE antibody (e.g., monoclonal antibody)
can be used to isolate a
TRADE polypeptide by standard techniques, such as affinity
chromatography or
immunoprecipitation. Anti- TRADE antibodies can facilitate the
purification of natural
TRADE polypeptides from cells and of recombinantly produced TRADE
polypeptides
expressed in host cells. Moreover, an anti- TRADE antibody can
be used to detect a
TRADE protein (e.g., in a cellular lysate or cell supernatant).

Detection may be
1 5 facilitated by coupling (ie., physically linking
) the antibody to a detectable substance.

Accordingly, in one embodiment, an anti- TRADE antibody of the invention is labeled
with a detectable substance. Examples of detectable substances include various ,
enzymes, prosthetic groups, fluorescent materials, luminescent. . .

Accordingly, in one embodiment, anti-TRADE antibodies can be used, e.g.,
intracellularly to inhibit protein activity. The use of intracellular antibodies to inhibit
protein function in a cell is known in the art (see e.g., Carlson, J. R., 1988, MoL Cell.

In one embodiment, a recombinant expression vector is prepared which encodes
the antibody chains in a form such that, upon introduction of the vector into a cell, the
antibody chains are expressed as a functional antibody in an intracellular compartment
of the cell. For inhibition of TRADE activity according to the inhibitory methods of the
invention, an intracellular antibody that specifically binds the TRADE protein is
expressed in the cytoplasm of the cell. To pre-pare an intracellular antibody expression
vector, antibody light and heavy chain cDNAs encoding antibody chains specific for the
target protein of interest, e.g., TRADE, are isolated, typically from a hybridoma that
secretes a monoclonal antibody specific for the TRADE protein. Hybridomas secreting
anti- TRADE monoclonal antibodies, or recombinant anti- TRADE monoclonal
antibodies, can be prepared as described above. Once a monoclonal antibody specific
for TRADE protein has been identified (e.g., either a hybridoma-derived monoclonal
antibody or a recombinant antibody from a combinatorial library), DNAs encoding the
light and heavy chains of the monoclonal antibody are isolated by standard molecular
biology techniques. For hybridoma derived antibodies, light and heavy chain cDNAs
can be obtained, for example, by PCR amplification or cDNA library screening. For
recombinant antibodies, such as from a phage display library, cDNA encoding the light
and heavy chains can be recovered from the display package (e.g., phage) isolated during
the library screening process. Nucleotide sequences of antibody light and heavy chain
genes from which PCR primers or cDNA library probes can be prepared are known in
the art. For. . .

Once obtained, the antibody light and heavy chain sequences are cloned into a
recombinant expression vector using standard methods. To allow for cytoplasmic
expression of the light and heavy chains, the nucleotide sequences

encoding the hydrophobic leaders of the light and heavy chains are removed. An intracellular antibody expression vector can encode an intracellular antibody in one of several different forms. For example, in one embodiment, the vector encodes full-length antibody light and heavy chains such that a full-length antibody is expressed 1 0 intracellularly. In another embodiment, the vector encodes a full-length light chain but only the VH/CH1 region of the heavy chain such that a Fab fragment is expressed intracellularly. In the most preferred embodiment., the vector encodes a single chain antibody (scFv) wherein the variable regions of the light and heavy chains are linked by a flexible peptide linker (e.g., (Gly4Serb) and expressed as a single chain molecule. To 1 5 inhibit TRADE activity in a cell, the expression vector encoding the anti- TRADE intracellular antibody is introduced into -the cell by standard transfection methods, as discussed herein.

An antibody or antibody portion of the invention can be derivatized or linked to another functional molecule (e.g., a peptide or polypeptide). Accordingly, the antibodies and antibody portions of the invention are intended to include derivatized and otherwise modified forms of the anti-TRADE antibodies described herein, including, e.g., antibodies conjugated to other molecules (e.g., antibodies or polypeptides which bind to other cell markers). For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., to create a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies).



Suitable crosslinkers include those that are heterobifurictional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine- I -napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, 1 0 horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction. . . agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody 1 5 may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

In one embodiment, anti-TRADE antibodies can be used to target cells expressing TRADE molecules. For example, an antibody can be used which recognizes a TRADE family molecules or which specifically recognizes a single TRADE family molecule and not another TRADE family molecule, e.g., an antibody which recognizes TRADEP. In one embodiment, such an antibody-toxin conjugate comprising the antibody and a toxin molecule can be used to deplete cells bearing a TRADE family or a specific TRADE molecule (e.g., by ablation). In a preferred embodiment, an anti-TRADE immunotoxin is used to target a tumor cell, e.g., in vivo or ex vivo. As used herein, the tenn toxin is meant to include molecules that are toxic. . .

A wide variety of toxins are known in the art and may be conjugated to the antibodies of the invention (see Hertler and Frankel, 1989, J Clin OncoL 7:1932-1942). . . . 1994, Nature Structural Biology 1:59-64), as does the sarcin-A toxin, derived from the mold Aspergillus giganteus (Lacadena et al., 1999, Proteins, 37:474-484). Antibody-toxin conjugates which include ricin-A and similar toxins have been described previously] in U.S. Patent Nos. 4,590,017, 4,906,469, 4,919,927, and 5,980,896, which are. . . . . diptheria toxin (from Corynebacterium diphtheriae) and inhibit protein synthesis (Foley et al., 1995, JBiol Chem, 270:23218-23225) can also be used in the antibody-toxin conjugates of the invention. Antibody-toxin conjugates which include



diptheria toxin or related toxins which ADP-ribosylate the EF-2 have been described

previously, e.g., in U.S. Patent Nos.. . .

62:361-363). Antibody-toxin conjugates which include maytansinoid have been described previously in U.S. Patent No. 5,208,020.

In one embodiment, a toxin for use in the antibody-toxin conjugates of the invention is an oligosaccharide. For example, the oligosaccharide calichearnicin is a bacterial product which was identified as one of a. . . . repair mechanisms (Chaudhry et al., 1999, Biochem Pharmacol, 57:531-538). Calicheamicin is a preferred toxin moiety for use in connection with the invention. Antibody calicheamicin conjugates have been described (Sievers et al., 1999, Blood, 93:3678-3684; Lode et al., 1998, Cancer Research, 58:2925-2928). Other synthetic cytotoxic t



compounds, such as CC- 1 065, have similar DNA-fragmenting mechanisms as calichearnicin and are also. . .

Pharmacol, 52:447-453). Antibody-toxin conjugates, in which calicheamicin is covalently attached to an antibody through disulfide bonds, have been described previously in U.S. Patent Nos. 5,773,001 and 5,739,116.

Molecular conjugates which include aerolysin have been described previously in U.S.

There are numerous methods known in the art, for conjugating a toxin to an antibody such that the activity of the toxin is appropriately delivered upon binding of the 1 5 antibody to a cell (Ghose and Blair, 1987, Crit Rev Ther Drug Carrier Syst, 3 ):263-3 59; Hermentin and Seiler, 1988, Behring. . . iWitt. ] 82:197 ). For example', when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifianctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in Monoclonal Antibody -Toxin Conjugates: Aiming the Magic Bullet, Thorpe et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference. The components may also be linked genetically (see, Chaudhary et al., 1989, Nature 339:394, which is herein incorporated by reference).

For example, in one embodiment, a covalent linkage can be formed between the antibody and the toxin. In some cases, the existing cell-binding portion of a toxin must first be removed or altered to suppress its non-specific activity

(Hertler and Frankel,
1989, J Clin Oncol 7:1932-1942). The covalent linkage of
antibody to toxin generally
involves formation of a thioester or a disuffide bond. For example,
conjugate
compounds can be prepared by using N-suceinimidyl
2(pyridyldithio)propionate,
which can generate a disulfide linkage between an
antibody and a toxin (Colombatti et
- 68 -
aL, 1983, JImmunology, 131:3091-3095). Numerous types of disulfide-bond
containing
linkers are known which can successfully be employed to
conjugate the toxin moiety
with a polypeptide. In one embodiment, linkers that contain a
disuffide bond that is
sterically hindered are preferred, due to their greater stability in
vivo, thus preventing
release of the toxin moiety prior to binding at the site of action.
Other methods forming
covalent linkages between have been described in U.S. Patent
Nos. 4,894,443,
5]208]021] 4,340,535, and EP 44167.

. . .

one or more regulatory sequences, selected on
the basis of the host cells to be used for expression, which is
operatively linked to the
micleic acid sequence to be expressed. Within a recombinant expression
vector,
operably linked is intended to mean that the nucleotide
sequence of interest is linked to
the regulatory sequence(s) in a manner which allows for expression of
the nucleotide
sequence (e.g., in an in vitro transcription/translation system. . .

. . .

proteins can be utilized in TRADE activity assays, (e.g., direct
assays or competitive assays described in detail below), or to generate
antibodies
specific for TRADE proteins, for example.

. . .

Publication No. WO 96/01313). Accordingly, in another
embodiment, the invention provides a recombinant expression vector in
which a TRADE
DNA is operatively linked to an inducible eukaryotic promoter,
thereby allowing for
inducible expression of a TRADE protein in eukaryotic cells.

. . .

a
DNA molecule of the invention cloned into the expression vector in an
antisense
orientation. That is, the DNA molecule is operatively linked
to a regulatory sequence in
a manner which allows for expression (by transcription of the DNA
molecule) of an
RNA molecule which is antisense to TRADE mRNA. Regulatory sequences
operatively
linked to a nucleic acid cloned in the antisense orientation
can be chosen which direct
the continuous expression of the antisense RNA. . .

. . .

be included in the transgene to increase the efficiency of expression of
the transgene. A tissue-specific regulatory sequence(s) can be operably
linked to a
TRADE transgene to direct expression of a TRADE protein to particular

cells. Methods
for generating transgenic animals via embryo manipulation. . .
. . .
kinase assays involve expressing the deletion constructs in a host cell, isolating the expressed protein protein, immunoprecipitating the expressed protein with
an antibody, and incubating the immune complex with 31p labeled ATP. This reaction is
then run on SDS-PAGE and autoradiographed. This method is. . .
. . .
blot analysis where the protein lysates are separated by SDS-PAGE, transferred to a membrane (i.e. nitrocellulose or nylon) and probed with an
antibody against a protein of interest. This method of detection is well known in the art.
. . .
of a TRADE molecule can be accomplished, e.g., by direct binding. In a direct
binding assay, the TRADE protein could be coupled with a radioisotope or enzymatic
label such that binding of the TRADE protein to a TRADE target molecule can be
determined by. . .
. . .
are also available for use
in the subject assay. For instance, either TRADE or its cognate binding protein can be
immobilized utilizing conjugation of biotin and streptavidin. For instance, biotinylated
TRADE molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using
techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford,
IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce
Chemical). Alternatively, antibodies reactive with TRADE but which do not interfere
with binding of upstream or downstream elements can be derivatized to the wells of the
plate, and TRADE trapped in the wells by antibody conjugation. As above, preparations
0 of a TRADE -binding protein and a test modulating agent are incubated in the TRADE -
presenting wells. . . be
quantitated. Exemplary methods for detecting such complexes, in addition to those
described above for the GST-immobilized complexes, include immunodetection of
complexes using antibodies reactive with the TRADE binding element, or which are
5 reactive with TRADE protein and compete with the binding element; as well as enzyme-
linked assays which rely on detecting an enzymatic activity associated with the binding
element, either intrinsic or extrinsic activity. In the instance of the latter, the enzyme can
be chemically conjugated or provided as a fusion protein with the TRADE -BP. To
illustrate, the TRADE -BP can be chemically cross-linked or genetically fused with
horseradish peroxidase, and the amount of protein trapped in the complex can be
assessed with a chromogenic substrate. . .

For processes which rely on immunodetection for quantitating one of the proteins
trapped in the complex, antibodies against the protein, such as anti- TRADE antibodies,
can be used. Alternatively, the protein to be detected in the complex can be epitope
tagged in the forra- of a fusion protein which includes, in addition to the TRADE
sequence, a second protein for which antibodies are readily available (e.g. from
commercial sources). For instance, the GST fusion proteins described above can also be
- 86 -
used for quantification of binding using antibodies against the GST moiety.. Other useful
epitope tags include myc-epitopes (e.g., see Ellison et aL, 1991, JBiol Chem 266:21150-
2115 7) which. . .

. . .
of TRADE are
known in the art (see discussions above). In one embodiment, within the expression
vector the TRADE-coding sequences are operatively linked to regulatory sequences that
allow for constitutive or inducible expression of TRADE in the indicator cell(s). Use of
a recombinant expression vector. . . that enhance or inhibit the activity of TRADE. In an alternative embodiment, within the expression vector the
TRADE coding sequences are operatively linked to regulatory sequences of the
endogenousTRADEgene(i.e.,thepromoterregulatoryregionderivedfromthe endogenous gene). Use of a recombinant expression vector in which TRADE expression
- 87 -
is controlled by. . .

. . .
transcription is altered by a modulation in TRADE expression or activity,
e.g., the 5' flanking promoter and enhancer regions, are operatively linked to a marker
1 5 (such as luciferase) which encodes a gene product,that can be readily detected.

. . .
catalytic/enzymatic activity of the target an
appropriate substrate, detecting the induction of a reporter gene (comprising a target-
responsive regulatory element operatively linked to a nucleic acid encoding a detectable
marker, e.g., chlorainphenicol acetyl transferase), or detecting a target-regulated cellular
I 0 response, e.g., apoptosis.. . . a epithelial cell. The hallmark of apoptosis is degradation of DNA. Early
in the process, this degradation occurs in intemucleosomal DNA linker regions. The
1 5 DNA cleavage may yield double-stranded and single-stranded DNA breaks. Apoptosis
cc-in be measured in cells using standard techniques.. . .

. . .
of a TRADE molecule can be accomplished, e.g., by direct binding. In a direct
binding assay, the TRADE protein could be coupled with a radioisotope or enzymatic
label such that binding of the TRADE protein to a TRADE target molecule

can be
determined by. . .
. . .
are also available for use
in the subject assay. For instance, either TRADE or its cognate binding protein can be
immobilized utilizing conjugation of biotin and streptavidin.
For instance, biotinylated
TRADE molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using
techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford,
IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce
Chemical). Alternatively, antibodies reactive with TRADE but which do not interfere
with binding of upstream or downstream elements can be derivatized to the wells of the
plate, and TRADE trapped in the wells by antibody
conjugation. As above, preparations
of a TRADE -binding.protein and a test modulating agent are incubated in the TRADE -
presenting wells of the. . . methods for detecting such complexes, in addition to those
- 95 -
described above for the GST-immobilized complexes, include immunodetection of
complexes using antibodies reactive with the TRADE binding element, or which are
reactive with TRADE protein and compete with the binding element; as well as enzyme-
linked assays which rely on detecting an enzymatic activity associated with the binding
element, either intrinsic or extrinsic activity. In the instance of the latter, the enzyme can
be chemically conjugated or provided as a fusion protein with the TRADE -BP. To
illustrate, the TRADE -BP can be chemically cross-linked or genetically fused with
horseradish peroxidase, and the amount of protein trapped in the complex can be
assessed with a chromogenic substrate. . .

For processes which rely on immunodetection for quantitating one of the proteins
1 5 trapped in the complex, antibodies against the protein, such as anti- TRADE antibodies
can be used. Alternatively, the protein to be detected in the complex can be epitope
tagged in the form of a fusion protein which includes, in addition to the TRADE
sequence, a second protein for which antibodies are readily available (e.g. from
commercial sources). For instance, the GST fusion proteins described above can also be
used for quantification of binding using antibodies against the GST moiety. Other useful
epitope tags include myc-epitopes (e.g., see Ellison et al., 1991, JBiol Chem 266:21150-
21157) which includes. . .
. . .
of TRADE are
known in the art (see discussions above). In one embodiment, within the expression

vector the TRADE-coding sequences are operatively linked to regulatory sequences that
allow for constitutive or inducible expression of TRADE in the indicator cell(s). Use of
1 5 a recombinant. . . that enhance or inhibit the
activity of TRADE. In an alternative embodiment, within the expression vector the
TRADE coding sequences are operatively linked to regulatory sequences of the
endogenous TRADE gene (i.e., the promoter regulatory region derived from the
endogenous gene). Use of a recombinant. . .
. . .
whose transcription is altered by a modulation in TRADE
expression or activity, e.g., the 5' flanking promoter and enhancer regions, are
operatively linked to a marker (such as luciferase) which
encodes a gene product that can
be readily detected.
. . .
catalytic/enzymatic activity of the target an
appropriate substrate, detecting the induction of a reporter gene (comprising a target-
responsive regulatory element operatively linked to a nucleic
acid encoding a detectable
marker, e.g., chloramphenicol acetyl transferase), or detecting a target-regulated cellular
-
response,, e.g., apoptosis. For example,. . . in a epithelial
cell. The hallmark of apoptosis is degradation of DNA. Early in the process, this
degradation occurs in intemucleosomal DNA linker regions. The
DNA cleavage may
yield double-stranded and single-stranded DNA breaks. Apoptosis can be measured in
cells using standard techniques. For example,. . .
. . .
of the transcription factor
are brought into close proximity. This proximity allows transcription of a reporter gene
(e.g., LacZ) which is operably linked to a transcriptional
regulatory site responsive to the
transcription factor. Expression of the reporter gene can be detected and cell colonies
containing. . .
. . .
model. For example, an
agent identified as described herein (e.g., a TRADE modulating agent, an antisense
TRADE nucleic acid molecule, a TRADE-specific antibody, or a
TRADE -binding
partner) can be used in an animal model to determine the efficacy, toxicity, or side
I 0 effects. . .

-
V. Other Uses and Methods of the Invention
The nucleic acid molecules, proteins, protein homologues, and antibodies
described herein can be used in one or more of the following methods: a) methods of
treatment, e.g., up- or down-modulating proliferation. . . protein or
production of TRADE protein forius which have
decreased or aberrant activity compared to TRADE wild type protein.

Moreover, the anti-TRADE antibodies of the invention can be used to detect and isolate TRADE proteins, regulate the bioavailability of TRADE proteins, and modulate a. . .

. . .

of a TRADE molecule in a sample, e.g., portions or fragments of the cDNA sequences identified herein (and the corresponding complete-gene sequences), antibodies that recognize TRADE family polypeptides or specific TRADE polypeptides, can be used in numerous ways to detect TRADE nucleic acid or polypeptide. . .

A preferred agent for detecting TRADE protein is an antibody capable of I 0 binding to TRADE protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')2) can be used. The term labeled, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well 1 5 as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term biological sample. . . for detection of TRADE mRNA include Northern hybridizations and in situ hybridizations. In vitro techniques for detection of TRADE protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitation and immunofluorescence. In vitro techniques for detection of TRADE genomic DNA include Southern hybridizations. Furthennore, in vivo techniques for detection of TRADE protein include introducing into a subject a labeled anti-TRADE antibody. -For-example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

. . .

may be performed, for example, by utilizing pre- I 0 packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a. . .

TRADE molecules can also be linked, conjugated, or administered with agents that provide desirable pharmaceutical or phannacodynarnic properties. For example, TRADE can be coupled to any substance known in the art to promote penetration or

transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection. (See for example, Friden et al., 1993, Science 259: 373-377 which is incorporated by reference). Furthermore, TRADE can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties.

. . .

be in a composition which aids in delivery into the cytosol of a cell. For example, a TRADE molecule may be conjugated with a carrier moiety such as a liposome that is capable of delivering the peptide into the cytosol of a cell. Such. . .

. . .

that measurement of the level of TRADE in a cell or cells such as in a group of cells, tissue or neoplasia, like will provide useful information regarding apoptotic state of that cell or cells. In addition, it can also be desirable to. . .

. . .

can include a reagent for determining expression of TRADE (e.g., a nucleic acid probe(s) for detecting TRADE mRNA or one or more antibodies for detection of TRADE proteins), a I 0 control to which the results of the subject are compared, and instructions for. . .

. . .

Tris-buffered saline solution (TB S) with IO% (w/v) normal swine serum for one hour. For non-liver sections, primary mAb was detected with biotin-conjugated goat anti-mouse/rabbit IgG (Dako) in TBS for 30 minutes. Staining was detected with streptABC complex/horseradish peroxidase (Dako) diluted 1: I 00 in. . .

. . .

of the four specimens. Focal immunoreactivity was also found in smooth muscle, and two cases showed weaker endothelial staining. The four prostate cancer specimens gave similar results, with strong, diffuse staining in the glandular epithelium. This signal was stronger, presumably as a result of the. . .

. . .

ducts (3+), and intense



panacinar cytoplasmic staining of hepatocytes (3+). In the hepatocellular carcinoma specimen, there was intense staining of tumor cells with both mAbs.

. . .

unlike TRADE, p75 NGF1 expression has been reported to be lost in malignant specimens and it is not expressed in metastatic tumor lines derived from the prostate. The growth inhibition mediated by the CD40 ligand on tumor cells may have therapeutic value (Hirano et al., 1999, Blood, 93:2999-3007).

Example 3. Immunochemical analysis of TRADE

A panel of twenty murine monoclonal antibodies (mAb) specific for the TRADE extracellular domain were prepared for analyzing TRADE protein expression.

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and adapted to grow in ascites. An affinity column. with immobilized Protein A (Pierce, Rockford, IL) was used to purify monoclonal antibody from ascites fluids. Antibody class and subclass were tested by using Mouse Hybridoma Subtyping kit as per manufacturer's instructions (Boehringer Mannheim, Indianapolis, IN).

. . .

Cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cells were stained with anti-TRADE or isotype matched control monoclonal antibodies at IO ptg/ml. Binding of primary antibody was detected with goat F(ablanti-murine IgG conjugated to biotin, followed by streptavidin-phycoerythrin (Southern Biotechnology Associates, Birmingham, AL).

. . .

a colonic adenocarcinoina, CaCo2. Specifically, the bottom panels show the results of treatment of a human astrocytoma cell line with both antibodies in the 1 5 presence and absence of TRADE-Fc fusion protein. The dotted lines represent the anti-TRADEoc #8 and # 1 6 (bottom left and bottom right panels, respectively). The solid lines represent the control mIgG 1 and the antibody (either #8 or # 1 6) in the presence of TRADE-Fc fusion protein. Specificity was confirmed by competing away the FACS staining. . . expression of TRADE in each of these cell lines was also confirmed by RT-PCR using TRADE specific primers. Two other prostate tumor cell lines, LNCaP.FGC and DU145, as well as other colon tumor lines, HCTI 16 and HT-29 were negative for TRADE expression by flow cytometry with these inAbs. Transiently transfected COS cells were analyzed. . .

. . .

once activated, enters the nucleus and activates transcription from several key genes involved in cell survival and proliferation checkpoints ( Karin, 1998, Cancer Jftom Scientific American, 4:92-99).

. . .

TRAF6 (Khursigara et al, 1999, J ofBiol Chernistry, 274:2597-2600). The p75GF also stimulates apoptosis, and a novel zinc finger containing protein, NRIF (neurotrophin receptor interacting protein) mediates this signal (Casademunt et al., 1999, EMBO Journal, 18:605 0-606 1). NRIF binds two motifs in the intracellular region. . .

. . .

of 50-60,000 Mr was noted, representing the
TRADE-Fc monomer. In non-reducing conditions, a species of approximately
120,000
Mr was noted, illustrating the disulphide-linked dinier form
of TRADE-Fc. This dirneric
forin is expected to be a potent, soluble antagonist of the TRADE
ligand.

. . .
in vitro kinase assays. Specifically, cDNAs encoding
Flag-tagged proteins (or vector control) were expressed in 293T cells
and lysates
immunoprecipitated using anti-Flag antibody or control
antibody. The immune-
-
complexes were subjected to kinase assays using 12p labelled ATP and
examined by
SDS-PAGE. The gels were dried and analyzed. . .

CLMEN 12 The method of claim 2, wherein the agent is an antibody
that recognizes a
TRADE family member polypeptide

24 The method of claim 15 or 16, wherein the agent is an
antibody that recognizes a
TRADE family polypeptide.

. . .
claim 29 or 30, wherein the disorder is a proliferative disease or
disorder selected from the group consisting of. inflammation and
neoplasia.

32 The method of claim 3 1, wherein the neoplasia is a
carcinoma.
I 0

33 The method of claim 3 1, wherein the neoplasia is present
in lung or prostate
tissue.

34 The method of claim 3 1, wherein the neoplasia is an
adenocarcinoma

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
L1 3555 S P75
L2 1651 S NEUROTROPHIN RECEPTOR
L3 806 S L1 AND L2
L4 775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5 108 S L3 AND L4
L6 490570 S ANTIBOD?
L7 6 S L6 AND L5
L8 2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9 2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10 420 S L9 AND L4
L11 95 S L10 AND L6
L12 21 S TARGET? AND L11
L13 1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14 26 S L13 AND L11
L15 8 S L14 AND L12
L16 1 S L15 NOT PY>2002
L17 3 S L14 NOT PY>2001

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L18          108 S INSULIN () GROWTH FACTOR RECEPTOR
L19          819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20          914 S L19 OR L18
L21            7 S TYPE 1 AND L18
L22          417 S L19 AND L4
L23           83 S L22 AND L6
L24           18 S L23 AND L13
L25            2 S L24 NOT PY>2001

     FILE 'REGISTRY' ENTERED AT 10:27:37 ON 10 MAY 2007
L26            0 S 5C3/CN
L27            0 S MC192/CN

     FILE 'PCTFULL' ENTERED AT 10:28:00 ON 10 MAY 2007
L28         1765 S P75
L29          407 S NEUROTROPHIN RECEPTOR
L30          208 S L28 AND L29
L31       109389 S CANCER? OR TUMOR? OR NEOPLAS?
L32        97772 S ANTIBOD?
L33        63932 S L32 AND L31
L34          322 S L29 AND L33
L35       568977 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L36          316 S L35 AND L34
L37          105 S L36 NOT PY>2001
L38           79 S L36 NOT PY>2000

=> s l29 or l28
L39         1964 L29 OR L28

=> s l39/clm or l39/ab
            321 NEUROTROPHIN/CLM
          23953 RECEPTOR/CLM
             30 NEUROTROPHIN RECEPTOR/CLM
                  ((NEUROTROPHIN(W)RECEPTOR)/CLM)
            172 P75/CLM
             62 NEUROTROPHIN/AB
             22 NEUROTROPHINS/AB
             78 NEUROTROPHIN/AB
                  ((NEUROTROPHIN OR NEUROTROPHINS)/AB)
          13885 RECEPTOR/AB
           4554 RECEPTORS/AB
          16605 RECEPTOR/AB
                  ((RECEPTOR OR RECEPTORS)/AB)
             13 NEUROTROPHIN RECEPTOR/AB
                  ((NEUROTROPHIN(W)RECEPTOR)/AB)
             33 P75/AB
L40          192 ((NEUROTROPHIN RECEPTOR/CLM) OR (P75/CLM)) OR ((NEUROTROPHIN
                 RECEPTOR/AB) OR (P75/AB))

=> s l40 and l33
L41          128 L40 AND L33

=> s l41 and l35
L42          127 L41 AND L35

=> s l42 not py>2000
        740172 PY>2000
L43           42 L42 NOT PY>2000

=> d kwic

L43    ANSWER 1 OF 42    PCTFULL   COPYRIGHT 2007 Univentio on STN
ABFR   L'invention concerne une molecule d'un acide nucleique isole codant pour
       un polypeptide
```

capable de lier un recepteur de p75¿NTR et une version purifiee dudit polypeptide capable de lier un recepteur de p75¿NTR. Cette invention se rapporte a un procede pour produire un polypeptide purifie capable de lier un recepteur de p75¿NTR, ainsi qu'a un oligonucleotide antisens comportant une sequence d'acide nucleique capable de s'hybrider specifiquement a une molecule d'ARNm codant le polypeptide ci-decrit. Elle decrit un procede pour produire un polypeptide capable de lier un recepteur de p75¿NTR a un vecteur approprie, ainsi qu'un procede pour provoquer l'apoptose, un procede pour determiner les effets physiologiques, un procede pour. . . HGR74 sous la forme d'un vecteur approprie, une composition pharmaceutique comprenant un polypeptide purifie capable de lier un recepteur de p75¿NTR et un excipient pharmaceutiquement acceptable et, enfin, un procede pour identifier un compose qui est un inhibiteur d'apoptose.

DETD

Figure 1E
Expression of endogenous NADE protein in SK-N-MC human neuroblastoma cells. SK-N-MC cell lysate treated with ALLN is immunoprecipitated by anti-NADE antibody, and subjected to immunoblotting by same antibody. Human NADE protein transiently expressed in 293T cells and untreated gels were used for controls. Heavy chain bands are resulted from antibodies using immunoprecipitation.

. . .
protein A wild type NADE, muNADE (Cys102Ser) , and muNADE (Cys121Ser) proteins transiently expressed in 293T cells were detected by immunoblotting with anti-NADE antibody. Transfection methods are described in material and methods. The cell lysate extracted from the 5293T cells transfected with parental vector was used. . .

. . .
NADE and p7 5NTR The cell lysates extracted from 293T cells co-transfected with Myc-tagged NADE and p7 5NTR were co-immunoprecipitated by anti-Myc antibody, and subjected to immunoblotting by anti-p75 NTR antibody. The 30 lysates from the cells transfected with each plasmid and a parental vector were used as controls. Transfection methods are described in. . .

Upper panel; Immunoprecipitates of anti-Myc antibody (IgGi) from each sample were subjected to immunoblotting analysis by anti p75 NTR antibody. Middle and lower panels indicated the NTR
5 expression level of p75 and NADE proteins by immunoblotting, respectively. The immunoprecipitate of anti-FLAG antibody (IgGl) was used as a control.

Figure 3E
35 Activation of Caspase-2 and 3 and degradation of PARP in co-transfected 293T cells. The cell extracts from 293T cells transfected by each cDNA as indicated were analyzed by immunoblotting with anti-Caspase-2, Caspase-3, and PARP antibody. The level of a-tubulin was measured as a control.

. . .
pM 2-mercaptoethanol (2-ME) for 5 min, subjected to a 12.5 % SDS-PAGE and analyzed by Western blot with the anti- -NADE polyclonal antibody. Df Interaction of NADE and its point mutants with p75NTR. The

interaction of mutants with p75NTR was measured by using the GST fusion. . .

. . .

described isolated nucleic acid
10 molecule encoding a polypeptide capable of binding a p75 NTR receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes. . .

. . .

This invention provides a vector which comprises the isolated 5nucleic acid encoding a polypeptide capable of binding a p75 NTR receptor, operatively linked to a promoter of RNA transcription. In an embodiment of the invention, where in the vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a p75 NTF] receptor,, 10 operatively linked to a promoter of RNA transcription is a plasmid. In another embodiment the above described isolated nucleic acid molecule which is a. . .

. . .

prototrophy to an
auxotrophic host, biocide resistance or resistance to heavy metals such as copper. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, 10 or introduced into the same cell by cotransformation.

This invention provides a monoclonal antibody directed to an epitope of a polypeptide capable of binding a p75 NTR receptor.

5In an embodiment the above described monoclonal antibody, said monoclonal antibody is directed to a mouse, rat or human polypeptide capable of binding a p75 NTR receptor.

The term antibody includes, by way of example, both 10 naturally occurring and non-naturally occurring antibodies.

Specifically, the term antibody includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term antibody includes chimeric antibodies,
wholly
synthetic antibodies, and fragments thereof. Optionally, an 15 antibody can be labeled with a detectable marker.
Detectable
markers include, for example, radioactive or fluorescent markers.

This invention provides a polyclonal antibody directed to an 20 epitope of the purified protein having the amino sequence as set forth in Figure 1G-1 (SEQ ID NO: -) . in a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a p75 11TR receptor, having the amino sequence as set forth in 25 Figure. . .

Polyclonal antibodies may be produced by injecting a host animal such as rabbit, rat, goat, mouse or other animal with the immunogen of this. . . polypeptide capable of binding a p75 NTR receptor. The
sera are extracted from the host animal and are screened to obtain polyclonal antibodies which are specific to the immunogen. Methods of screening for polyclonal antibodies 35 are well known to those of ordinary skill in the art such as those disclosed in Harlow & Lane, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratories, Cold Spring Harbor,

NY: 1988) the contents of which are hereby incorporated by reference.

5The monoclonal antibodies may be produced by immunizing for example, mice with an immunogen. The mice are inoculated intra-peritoneaily with an immunogenic amount of the. . .

In the practice of the subject invention any of the above-described antibodies may be labeled with a detectable marker.

15 In one embodiment, the labeled antibody is a purified labeled
antibody. As used in the subject invention, the term
antibody includes, but is not limited to, both naturally occurring and non-naturally occurring antibodies.

Specifically, the term antibody includes polyclonal and
20 monoclonal antibodies, and binding fragments thereof.

Furthermore, the term antibody includes chimeric antibodies
and wholly synthetic antibodies, and fragments thereof.

Furthermore, the term antibody includes chimeric antibodies
25 and wholly synthetic antibodies, and fragments thereof. A detectable moiety which functions as detectable labels are well known to those of ordinary skill in the art. . . The secondary enzymatic or binding step may
comprise the use of digoxigenin, alkaline phosphatase, horseradish peroxidase, 8-galactosidase, fluorescein or steptavidin/biotin. Methods of labeling antibodies are well
35 known in the art.

Determining whether the antibody forms such a complex may be accomplished according to methods well known to those skilled in the art. In the preferred embodiment,. . .

The antibody may be bound to an insoluble matrix such as that used in affinity chromatography. As used in the subject invention, isolating the cells which form a complex with the immobilized monoclonal antibody may be achieved by standard methods well known to those skilled in the art. For example, isolating may comprise affinity chromatography using immobilized antibody.

Alternatively, the antibody may be a free antibody. In this
15 case, isolating may comprise cell sorting using free, labeled primary or secondary antibodies. Such cell sorting methods are standard and are well known to those skilled in the art.

The labeled antibody may be a polyclonal or monoclonal
antibody. In one embodiment, the labeled antibody I .s a
purified labeled antibody. The term antibody includes, by
way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term antibody includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term antibody includes chimeric
antibodies and wholly synthetic antibodies, and fragments
thereof. The detectable marker may be, for example,

radioactive or fluorescent. Methods of labeling antibodies
30 are well known in the art.

. . .
of the above described transgenic nonhuman
15 mammal, the DNA encoding a polypeptide capable of binding a
p75 IT' receptor is operatively linked to tissue specific
regulatory elements.

An apoptosis inducing compound is defined as a compound which
may be, but not limited to, antibodies, inorganic compounds,
organic compounds, peptides, peptidomimetic compounds,
10 polypeptides or proteins, fragments or derivatives which
share some or all properities, e.g. fusion proteins,. . .

. . .
mammalian
homologs of the Caenorhabiditis elegans death gene ced-3,
which are required for mammalian apoptosis. Increased levels
of caspase-2 and caspase-3 have been linked to apoptosis.

. . .
a DNA molecule. In an embodiment of the
above described transgenic nonhuman mammal, the DNA encoding
a human HGR74 protein is operatively linked to tissue
specific regulatory elements.

. . .
wherein the compound is bound to a
solid support. In an embodiment of the above described
method, wherein the compound comprises an antibody, an
5inorganic compound, an organic compound, a peptide, a
peptidomimetic compound, a polypeptide or a protein. In an
embodiment of the above described. . .

. . .
cytoplasmic protein may be bound to a solid
support. Also the compound may be bound to a solid support
and comprises an antibody, an inorganic compound, an organic
compound, a peptide, a peptidomimetic compound, a polypeptide
or a protein.

. . .
the compound is bound to a
2 0solid support. In an embodiment of the above described
method, wherein the compound comprises an antibody, an
inorganic compound, an organic compound, a peptide, a
peptidomimetic compound, a polypeptide or a protein. In an
embodiment of the above described. . .

. . .
cytoplasmic protein may be bound to a solid
support. Also the compound may be bound to a solid support
and comprises an antibody, an inorganic compound, an organic
compound, a peptide, a peptidomimetic compound, a polypeptide
or a protein.

Experimental Details
Results and Discussions
The p75 IITR is the first-isolated neurotrophin receptor and the
member of TNFR (tumor necrosis factor receptor) family (7,
8). However, its functional role and signaling pathway has
remained largely unclear (9) . The existence of. . .

. . .
of the alternative
splicing form. The endogenous NADE protein was also
confirmed in human neuroblastoma cell line, SK-N-MC by
immunoprecipitation using the anti-NADE antibody (Fig. 1e).

. . .
NADE protein
5can be detected only in the presence of the ubiquitin

inhibitor such as ALLN, suggesting that NADE is modified by ubiquitin conjugating system leading to subsequent degradation by the proteasome. The molecular size of NADE is estimated to 22 kDa by the SDS-PAGE, and. . .

Antibodies. The polyclonal anti-NADE antibody was prepared
by immunization of GST-mouse NADE fusion protein into the rabbit. The NADE specific antibody was affinity purified by antigen coupled Sepharose 4B. The polyclonal anti-rat p75 ITI was kindly gifted from Dr. M. V. Chao. The monoclonal anti-Myc antibody (9E10) was purchased from BIOMOL. The polyclonal anti-Caspase-3 antibody (H-277) was purchased from Santa Cruz Biotechnology. The polyclonal Caspase-2 antibody was kindly gifted from Dr. Lloyd A. Greene. HRP conjugated anti-rabbit IgG was purchased from Bio-Rad.

. . .
lysed in 0.5 ml of RIPA buffer. The
supernatant of centrifuge (100,000 x g) was mixed with 1 Ag of polyclonal anti-NADE antibody coupled Sepharose 4B, and
incubated for 4 hours at 4 OC. After washing, the gels were boiled by 30 Al of SDS-PAGE sampling buffer and subjected to 12.5 % of SDS-PAGE. Immunoblotting was performed by polyclonal anti-NADE antibody (2 Ag/ml). In Fig. 1ff 10 Ag 35 of cell lysate extracted from each transfected 293T cells were used for the detection. . .

. . .
[35S] methionine labeled,
and in vitro- translated NADE protein was mixed with 5 Al of GST-rat p75 NTRI CD fusion protein or GST-coupled GSH-Sepharose
4B (Pharmacia) in 100 Al of NETN buffer (20 mM Tris-HC1 pH 8.01 100 mM NaCl, 1 mM EDTA, 0.2 %. . .

. . .
cells by
were lysed in 1 ml of NETN buffer and centrifuged (100,000 Ag). The supernatants were immunoprecipitated by 2 Ag of anti-Myc antibody coupled Protein G Sepharose 4B (Pharmacia)
for 2 hours at 4 'C. Following the 5 times washing, gels were subjected to 7.5 % SDS-PAGE, and Western blot analysis by rabbit polyclonal anti-p75 NTR antibody.

12, Smith, R. A. & Baglioni, C. The active form of tumor necrosis factor is a trimer. J. Biol. Chem. 262, 6951-6954 (1987).

. . .
normal development or in -response to a variety of stimuli, including DNA damage, growth factor deprivation, and abnormal expression of oncogenes or tumor suppressor genes (1-3). Apoptosis induced by these various reagents appears to be mediated by a common set of downstream elements that act as. . . as well as knockout and transgenic mice (5-7). However, the molecular mechanism of pro-apoptotic signaling involved in p75NTR is not well characterized. Recently, tumor necrosis factor receptor-associated factor (TRAF) family proteins, FAP-1, and zinc finger proteins have been reported to interact with p75NTR (ICD) (8-12) However,. . .

Reagents and Antibodies. Mouse nerve growth factor (NGF) was obtained from Sigma. TO-PRO-3 iodide was obtained from Molecular Probes. The anti-ot-NADE polyclonal antibody was

prepared as described previously (10).

In vi tro binding assay. In vitro-translated [35si methionine-labeled proteins were generated by using the TNT-coupled reticulocyte lysate system (Promega). Binding 5 assay was performed as described previously (22).

. . .

reducing condition, suggesting that NES is necessary for self-association of NADE and that the regulation of nuclear export of NADE may be linked to the association or dissociation of NADE monomer. Three of the key hydrophobic residues of NES, Leu 94, Leu 97 and Leu 99,. . .

10) . The expression of endogenous NADE was confirmed by anti-o(-NADE antibody in 293T, PC12 and nnr5 cells (data not shown). Introduction of NADE mutants, N (1-60), N (1-120) and N (C121S) with. . .

. . .

NADE NES motif is crucial for those functions. NES motif has been reported to require for self-association of p53 and may be linked to regulation of subcellular localization and p53 activity (30). NADE NES may be also important for the regulation of sublocalization recruitment to p75NTR. . .

8. The isolated nucleic acid of claim 1 operatively linked to a promoter of RNA transcription.

CLMEN. . . 11 The isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule encodes human or mouse polypeptide capable of binding p75 NTR receptor.

12 The isolated nucleic acid molecule of claim 11, wherein the nucleic acid molecule encodes a polypeptide capable of binding p75 NTR receptor set forth in Figure 1G-1 (SEQ ID NO: -) -

13 The isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule encodes a polypeptide capable of binding p75 NTR receptor.

14 The isolated nucleic acid molecule of claim 9 wherein the polypeptide capable of binding p75 NTR receptor is mouse, rat or human protein.

18 A method of producing a polypeptide capable of binding p75 NT' receptor which comprises growing the host cells of claim 17 under suitable conditions permitting production of the polypeptide.

. . .

An antisense oligonucleotide having a nucleic acid sequence capable of specifically hybridizing to an mRNA molecule encoding a polypeptide capable of binding p75 ITR receptor.

29 A purified polypeptide capable of binding p75 NTR receptor.

30 A purified polypeptide capable of binding p75 NTR receptor encoded by the isolated nucleic acid of claim 1.

31 A purified unique polypeptide fragment of the polypeptide capable of binding p75 NTR receptor of claim

3 0 .

. . .

the same amino acid
sequence as set forth in Figure 1G-1 (SEQ ID NO: -).
3 3 The polypeptide capable of binding p75 NTR receptor of claim 30 having the amino acid sequence as set forth in Figure 1G-1 (SEQ ID NO:

34 The polypeptide capable of binding p7 5NT' receptor of claim 33 which is a vertebrate polypeptide capable of binding p75 NTR receptor.

. . .

37 The polypeptide of claim 35 which comprises an amino acid sequence of NCLRILMGELSN.
3 8 .The vertebrate polypeptide capable of binding p75 NTR receptor of claim 34 which is a mouse, rat, or human polypeptide capable of binding p75 NTR receptor.

39 A monoclonal antibody directed to an epitope of a polypeptide capable of binding p75 NTR receptor of claim 35.

40 A monoclonal antibody of claim 33 directed to a mouse, rat or human polypeptide capable of binding p75 NTR receptor.

41 A polyclonal antibody directed to an epitope of the polypeptide capable of binding p75 NTR receptor of claim 32.

42 A polyclonal antibody of claim 41 directed to a mouse, rat or human polypeptide capable of binding p75 ITI receptor.

43 A method of inducing apoptosis in cells which comprises expressing a polypeptide capable of binding p75 NTR receptor in the cells.

47 The transgenic nonhuman mammal of claim 46, wherein the DNA encoding a polypeptide capable of binding p75 ITI receptor is operatively linked to tissue specific regulatory elements.

48 A method of determining physiological effects of expressing varying levels of a polypeptide capable of binding p75 IITR receptor in a transgenic nonhuman mammal which comprises producing a panel of transgenic non human mammal expressing a different amount of polypeptide capable of binding p75 NTR receptor.

49 A method of producing a polypeptide capable of binding p75 NTR receptor into a suitable vector which comprises.:
(a) inserting a nucleic acid molecule encoding the polypeptide capable of binding p75 NTR receptor into a suitable vector;
(b) introducing the resulting vector into a suitable host cell;
(c) selecting the introduced host cell for the expression of the polypeptide capable of binding p75 NTR receptor;
(d) culturing the selected cell to produce the polypeptide capable of binding p75 NTR receptor; and
(e) recovering the polypeptide capable of binding p75 NTR receptor produced.

50 A method of inducing apoptosis of cells in a subject comprising administering to the subject a purified polypeptide capable of binding p75 NTR receptor in an amount effective to induce apoptosis.

53 A pharmaceutical composition comprising a purified polypeptide capable of binding p75 NTR receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

54 A pharmaceutical composition comprising an effective amount of a purified Polypeptide capable of binding p75 IT' receptor of either claim 32 or 33 and a pharmaceutically acceptable carrier.

55 A method of identifying a compound capable of inhibiting binding between p75 NTR receptor and a polypeptide capable of binding p75 NTR receptor comprising:
a) contacting the compound with the polypeptide capable of binding to p75 ITI receptor under conditions permitting the binding of the Polypeptide capable of binding to p75 IT' receptor and p7 SNTR receptor to form a complex;
NTR
b) contacting the p75 receptor with the mixture from step a); and
C) measuring the amount of the formed complexes or the unbound p75 ITI receptor or the unbound polypeptide or any combination thereof.

56 A method of identifying a compound capable of inhibiting binding between p75 NTR receptor and a polypeptide capable of binding p75 NTR receptor, where said binding forms a complex between p75 IT' receptor and a polypeptide capable of binding p75 NTR receptor, comprising:
a) contacting the compound with the p75 ITR receptor under conditions permitting the binding of the polypeptide capable of binding to p75 NTR receptor and p75 NTR receptor to form a complex;
b) contacting the p75 NTR receptor with the mixture from step a); and
C) measuring the amount of the formed complexes or the unbound p75 NTR receptor or the unbound polypeptide or any combination thereof.
20 57. The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a neurotrophin associated cell death executor.

58 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a human HGR74 protein.

59 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a musnade3a sequence as defined on Figure 1H.

60 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a hunade3a1 sequence as defined on Figure 1H.

61 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 ITR receptor is a hunade3a2 sequence as defined on Figure 1H.

62 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3a sequence as defined on Figure 1H.

63 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a ratnad3b sequence as defined on Figure 1H.

64 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a musnade3b sequence as defined on Figure 1H.

65 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 IT' receptor is a humnadel sequence as defined on Figure 1H.

66 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a ratnadel sequence as defined on Figure 1H.

67 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 NTR receptor is a musnadel sequence as defined on Figure 1H.

68 The method of claims 55 or 56 wherein the polypeptide capable of binding p75 ITR receptor is a humnade2 sequence as defined on Figure 1H.

compound
comprising:
a) contacting a subject with an appropriate amount of the compound; and
b) measuring the expression level of a polypeptide capable of binding p75 NTR receptor gene and p75 14TR
gene in the subject, an increase of the expression levels of a polypeptide capable of binding p75 NTR receptor gene and p75 NTR gene indicating that the compound is an apoptosis inducing compound.

72 A method for identifying an apoptosis inducing compound comprising:
a contacting a cell with an appropriate amount of the compound; and
b) measuring the expression level of a polypeptide capable of binding a p75 NTR receptor gene and p75 NTR
gene in the cell, an increase of the expression levels of polypeptide capable of binding p75 NTR receptor gene and p75 NTR gene indicating that the compound is an apoptosis inducing compound.

73 A method for screening cDNA libraries of a polypeptide capable of binding p75 NTR receptor using a yeast two-hybrid system using a p75 NTR intracellular domain as a target.

76 The method of claim 73 where the p75 NTR intracellular domain target is mammalian.
35 77. The method of claim 76 where the mammalian p75 NTR intracellular domain target is a rat, mouse or human
p75 ITI intracellular domain target.

and caspase-3 activity to
cleave poly (ADP-ribose) polymerase and fragment
nuclear DNA in a cell by co-expression of polypeptide
NTR NTI
capable of binding p75 receptor and p75

79 A method to inhibit NF-KB activation in a cell with
NTR NTR
polypeptide capable of binding p75 receptor and p75

80 A method to detect a neurodegenerative disease- in a
subject by detecting expression levels of a polypeptide
NT NTII
capable of binding p75 receptor and p75

84 The transgenic nonhuman mammal of claim 83 where the DNA
encoding a human HGR74 protein is operatively linked to
tissue specific regulatory elements.

. . .
compound
comprising:
(a) contacting a subject with an appropriate amount of
the compound; and
(b) measuring the expression level of human HGR74
protein gene and p75 NTR gene in the subj ect, an
increase of the expression levels of human HGR74
protein and p75 NTR gene indicating that the compound
is an apoptosis inducing compound.

. . .
compound
comprising:
(a) contacting a cell with an appropriate amount of
the compound; and
(b) measuring the expression level of human HGR74
protein gene and p75 NTR gene in the cell, an
increase of the expression levels of human HGR74
protein gene and p75 NTR gene indicating that the
compound is an apoptosis inducing compound.

95 A method for screening cDNA libraries human HGR74
protein using a yeast two-hybrid system using a p75 NTR
intracellular domain as a target.

98 The method of claim 95 where the p75 NTR intracellular
domain target is mammalian.

99 The method of claim 98 where the mammalian p7 SITR
intracellular domain target is a rat, mouse or human
p75 NTR intracellular domain target.
100. A method to induce caspase-2 and caspase-3 activity to
cleave poly (ADP-ribose) polymerase and fragment
nuclear DNA in a cell by co-expression of human HGR74
NTR
protein and p75
101. A method to inhibit NF-KB activation in a cell with
NTR
human HGR74 protein and p75
102. A method to detect a neurodegenerative disease in a
subject by detecting expression levels of human HGR74
NTR
protein and p75
103. The method of claim 102 wherein the subject is a mammal.
104. The method of claim 103, wherein the mammal is. . . method of
identifying a compound, which is an

apoptosis inhibitor, said compound is capable of
inhibiting specific binding between polypeptide capable
of binding p75 ITR receptor and p75 NTR receptor, so
as to
prevent apoptosis which comprises:
(a) contacting the polypeptide capable of binding
p75 IT' receptor with a plurality of compounds
under conditions permitting binding between a
is known compound previously shown to be able to
displace a polypeptide capable of binding
p75 NTR receptor and the p75 NTF' receptor and the
bound p75 NTR receptor to form a complex; and
(b) detecting the displaced polypeptide capable
of binding p75 11TR receptor or the complex
formed in step (a), wherein the displacement
indicates that the compound is capable of
inhibiting specific binding between the
polypeptide capable of binding p75 11TR receptor
and the p75 NTR receptor.
106. The method of claim 105, wherein the inhibition of
specific binding between the polypeptide capable of
binding p75 NTR receptor and the p75 NTR receptor
affects
the transcription activity of a reporter gene.
107. The method of claim 106, where in step (b) the displaced
polypeptide capable of binding p75 NTR receptor or the
complex is detected by comparing the transcription
activity of a reporter gene before and after the
contacting with the compound in step (a), where a change
of the activity indicates that the specific binding
between the polypeptide capable of binding p75 NTR
0 0
receptor and the p75 NTR receptor is inhibited and the
polypeptide capable of binding p75 NTR receptor is
displaced.
108. The method of claim 105, wherein the p75 NTR receptor is
bound to asolid support.
109. The method of claim 105, wherein the compound is bound
to a solid support.
110. The method of claim 105, wherein the compound comprises
an antibody, an inorganic compound, an organic compound,
a peptide, a peptidomimetic compound, a polypeptide or
a protein.
111. The method of claim 105 wherein. . . wherein the contacting or
step
(a) is in a mammalian cell.
115. The method of claim 105, wherein the polypeptide capable
of binding p75 NTR receptor is a cell surface receptor.
116. The method of claim 112, wherein the cell-surface
receptor is the p75 receptor.
117. The method of claim 105 where in the polypeptide capable
of binding p75 NTR receptor is a neurotrophin associated
cell death exectuor.
118. The method of claim 105 where in the polypeptide capable
of binding p75 IT' receptor is a human HGR74 protein.
119. The method of claim 105 wherein the polypeptide capable
of binding p75 ITR receptor is a neurotrophin associated
cell death executor.
120. The method of claim 105 wherein the polypeptide capable
of binding p75 NTR receptor is a human HGR74 protein.
121. The method of claim 105 wherein the polypeptide capable
of binding p75 NTR receptor is a musnade3a sequence as
defined on Figure 1H.
122. The method of claim 105 wherein the polypeptide capable

of binding p75 NTR receptor is a hunade3a1 sequence as defined on Figure 1H.
123. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a hunade3a2 sequence as defined on Figure 1H.
124. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3a sequence as defined on Figure 1H.
125. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a ratnad3b sequence as defined on Figure 1H.
126. The method of claim 105 wherein the polypeptide capable of binding p75 11TR receptor is a musnade3b sequence as defined on Figure 1H.
127. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a humnadel sequence as defined on Figure 1H.
128. The method of claim 105 wherein the polypeptide capable of binding p75 ITR receptor is a ratnadel sequence as defined on Figure 1H.
129. The method of claim 105 wherein the polypeptide capable of binding. . . SIT' receptor is a musnadel sequence as defined on Figure 1H.
130. The method of claim 105 wherein the polypeptide capable of binding p75 NTR receptor is a humnade2 sequence as defined on Figure 1H.
131. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin is associated cell death executor protein (NADE), wherein the N-terminal 1-40 amino acids of wild type NADE polypeptide have. . . presence of p75NTR-
132. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 72-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-71), and the NADE N(1-71) induces apoptosis in the presence of p75 NTR and in the absence of NTR
p75
133. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the N-terminal 1-40 amino acids and the C-terminal 72-124 amino acids. . . of p7 5NTR.
135. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 113-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-112) and the NADE N(1-112) induces NTR
apoptosis in the presence of p75
136. An isolated nucleic acid molecule encoding a deletion mutant of a wild type polypeptide capable of binding with a p75 NTR receptor, designated neurotrophin associated cell death executor protein (NADE), wherein the C-terminal 101-124 amino acids of wild type NADE polypeptide have been deleted and the deletion mutant is designated NADE N(1-100) and the NADE N(1-100) induces

apoptosis in the presence of p75 NTR and in the absence of
NTR
p75
137. An isolated nucleic acid molecule encoding a mutation of
NTR
a wild type polypeptide capable of binding with a p75
receptor, designated neurotrophin associated cell death
executor protein (NADE) , wherein the point mutation
results in Ala at amino acid position 99 for. . . wild type NADE
polypeptide,
wherein the substitution mutant polypeptide is
designated NADE N(L99A) and the NADE N(L99A) induces
ITI
apoptosis in the presence of p75 .
Mouse HADE 1 MANVHQENEEMFQPLQNGEEDRPVGGGEGHQPAGNNNNNNHNHNM
Human NADE 1 MANIHQENEEMEQPMQNGEEDRPLGGGEGHQPAGN ----------
Mouse NADE 51 GQARRLAPNFRWAIPNRQMNDGLGGDGDDMEMFMEEMREIER:K::L]RE
Human NADE 38 GQARRLAPNFRWAIPNRQINDGMGGDGDDMEIFMEEMREIRRKLRE
v
Mouse NADE 101 E]L] ]]HDHHDEFCLMP 124
Human NADE. . . NHYD ---- FCLIP 97
humadel IFMEEMREIRRKLRE LQLRNCLRI124GELS NHHDHHDEFICLMP
ratnadel MFMEEMREIRRKLRE LQLRNCLRILMGELS NHHDHHDEFCLMP
musnadel EF7MEMREIRRKLRE LQLRNCLRILMELS NHHDHHDEFCLMP
hunmade2 RFMEEMRELRRKIRE LQLRYSLRILIGDPP -HHDBHDEFCLMP
Figure IH
/29
Figure 2A
kD
48
3 4
28
NADE
21
Figure 2B
4;]
Y.
N
kD
103
77 p75 NTR
48 mm]
344 .9*- rotein G
/29
NGF
(ng/ml)
0 10 100
IP: myc
4-p75NTR
WB:p75NTR
WB:p75NTR A=p75NTR
WB:myc .4w NADE monomer
Figure 2 C
/29
Control NADE
p75NTR NADE + p75NTR
Figure 3 A
/29
Figure. . . 8A
/29
GFP TO-PRO3
GFP-vector
GFP-NADE (WT.)
GFP-N (L99A)

GFP-N (L94A, L97A, L99A)
FIGURE 8B
/29
plcl]
q
]N
2-ME: ] + ] + ] +
84,0
41.0 -o* Dimer
31,7
-- A* Monomer
1819
FIGURE 8C
/29
Oj
Se
OQS #4]
C,
*4w
Oj Oj
40
GST-p75 (ICD) + +
GST + + +
NOWN
FIGURE 8D
/29
40
30
20
CL
0
CL
lom
0
CP Jjcl
CN
FIGURE 8E
/29
M NGF
60- MNGF +
40-
20-
4)
T
0
p75NTR + + +
MNADE MIMI,
FIGURE 9A
F-IGURE 9C
FIGURE 9B PC. . .

=> d his

(FILE 'HOME' ENTERED AT 10:19:49 ON 10 MAY 2007)

FILE 'CAPLUS' ENTERED AT 10:20:15 ON 10 MAY 2007
L1 3555 S P75
L2 1651 S NEUROTROPHIN RECEPTOR
L3 806 S L1 AND L2
L4 775765 S CANCER? OR TUMOR? OR NEOPLAS?
L5 108 S L3 AND L4
L6 490570 S ANTIBOD?
L7 6 S L6 AND L5

```
L8          2288 S TRKA OR NEUTROPHILIC RECEPTOR TYROSINE KINASE
L9          2288 S TRKA OR (NEUTROPHILIC RECEPTOR TYROSINE KINASE)
L10          420 S L9 AND L4
L11           95 S L10 AND L6
L12           21 S TARGET? AND L11
L13      1486130 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L14           26 S L13 AND L11
L15            8 S L14 AND L12
L16            1 S L15 NOT PY>2002
L17            3 S L14 NOT PY>2001
L18          108 S INSULIN () GROWTH FACTOR RECEPTOR
L19          819 S (IGF-1R) OR (IGF1R) OR (IGF 1R)
L20          914 S L19 OR L18
L21            7 S TYPE 1 AND L18
L22          417 S L19 AND L4
L23           83 S L22 AND L6
L24           18 S L23 AND L13
L25            2 S L24 NOT PY>2001

     FILE 'REGISTRY' ENTERED AT 10:27:37 ON 10 MAY 2007
L26            0 S 5C3/CN
L27            0 S MC192/CN

     FILE 'PCTFULL' ENTERED AT 10:28:00 ON 10 MAY 2007
L28         1765 S P75
L29          407 S NEUROTROPHIN RECEPTOR
L30          208 S L28 AND L29
L31       109389 S CANCER? OR TUMOR? OR NEOPLAS?
L32        97772 S ANTIBOD?
L33        63932 S L32 AND L31
L34          322 S L29 AND L33
L35       568977 S IMMUNOCONJUGATE OR (CONJUGAT? OR LINK? OR COUPL?)
L36          316 S L35 AND L34
L37          105 S L36 NOT PY>2001
L38           79 S L36 NOT PY>2000
L39         1964 S L29 OR L28
L40          192 S L39/CLM OR L39/AB
L41          128 S L40 AND L33
L42          127 S L41 AND L35
L43           42 S L42 NOT PY>2000

=> s l43 and dox? or (paclitaxel or taxol or taxane)
          19784 DOX?
           8111 PACLITAXEL
             66 PACLITAXELS
           8113 PACLITAXEL
                  (PACLITAXEL OR PACLITAXELS)
           8165 TAXOL
            240 TAXOLS
           8235 TAXOL
                  (TAXOL OR TAXOLS)
           1713 TAXANE
           2293 TAXANES
           3137 TAXANE
                  (TAXANE OR TAXANES)
L44       13134 L43 AND DOX? OR (PACLITAXEL OR TAXOL OR TAXANE)

=> s l43 and (dox? or (paclitaxel or taxol or taxane))
          19784 DOX?
           8111 PACLITAXEL
             66 PACLITAXELS
           8113 PACLITAXEL
                  (PACLITAXEL OR PACLITAXELS)
           8165 TAXOL
```

```
          240 TAXOLS
         8235 TAXOL
                (TAXOL OR TAXOLS)
         1713 TAXANE
         2293 TAXANES
         3137 TAXANE
                (TAXANE OR TAXANES)
L45         3 L43 AND (DOX? OR (PACLITAXEL OR TAXOL OR TAXANE))

=> d ibib 1-3

L45    ANSWER 1 OF 3     PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:        2000073321 PCTFULL  ED 20020515
TITLE (ENGLISH):         HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10
TITLE (FRENCH):          TR10, RECEPTEUR DE FACTEUR DE NECROSE TUMORALE
                         HUMAIN
INVENTOR(S):             ROSEN, Craig, A.;
                         NI, JianRP : KLEIN, Jonathan, L.
PATENT ASSIGNEE(S):      HUMAN GENOME SCIENCES, INC.;
                         ROSEN, Craig, A.;
                         NI, Jian
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 2000073321         A1 20001207
DESIGNATED STATES
       W:                AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE
                         DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE
                         KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX
                         NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA
                         UG US UZ VN YU ZA ZW GH GM KE LS MW MZ SD SL SZ TZ UG
                         ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI
                         FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN
                         GW ML MR NE SN TD TG
PRIORITY INFO.:          US 1999-60/136,786      19990528
                         US 1999-60/142,563      19990707
                         US 1999-60/144,023      19990715
APPLICATION INFO.:       WO 2000-US14554       A  20000526

L45    ANSWER 2 OF 3     PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:        1999045944 PCTFULL  ED 20020515
TITLE (ENGLISH):         PROAPOPTOTIC PEPTIDES, DEPENDENCE POLYPEPTIDES AND
                         METHODS OF USE
TITLE (FRENCH):          PEPTIDES PROAPOPTOTIQUES, POLYPEPTIDES DE DEPENDANCE,
                         ET MODE D'UTILISATION
INVENTOR(S):             BREDESEN, Dale, E.;
                         RABIZADEH, Shahrooz
PATENT ASSIGNEE(S):      THE BURNHAM INSTITUTE
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
                         ---------------------------------
                         WO 9945944            A1 19990916
DESIGNATED STATES
       W:                AU CA JP AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC
                         NL PT SE
PRIORITY INFO.:          US 1998-09/041,886      19980312
APPLICATION INFO.:       WO 1999-US5250        A  19990311

L45    ANSWER 3 OF 3     PCTFULL   COPYRIGHT 2007 Univentio on STN
ACCESSION NUMBER:        1994022866 PCTFULL  ED 20020513
```

TITLE (ENGLISH): PYRAZOLOQUINAZOLONE DERIVATIVES AS NEUROTROPHIC AGENTS
TITLE (FRENCH): DERIVES DE LA PYRAZOLOQUINAZOLONE EN TANT QU'AGENTS NEUROTROPES
INVENTOR(S): JAEN, Juan, Carlos;
CAPRATHE, Bradley, William
PATENT ASSIGNEE(S): WARNER-LAMBERT COMPANY
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

| NUMBER | KIND | DATE |
|---|---|---|
| WO 9422866 | A1 | 19941013 |

DESIGNATED STATES
W: AU CA CZ FI HU JP KR NO NZ RU SK AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
PRIORITY INFO.: US 1993-8/038,374 19930329
APPLICATION INFO.: WO 1993-US6617 A 19930714

=> d kwic 2

L45 ANSWER 2 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN

DETD . . . factors,
extracellular matrix, CD40 ligand, viral gene products, zinc, estrogen and androgens. In contrast, stimuli which promote apoptosis include growth factors such as tumor necrosis factor (TNF), Fas, and transforming growth factor ] (TGF]), growth factor withdrawal, loss of extracellular matrix attachment, intracellular calcium and glucocorticoids, for example.. . .
. . .
cellular apoptosis. Thus,
the peptides are useful for the treatment of various pathological conditions characterized by unregulated cell growth or survival such as cancer, autoimmune and fibrotic disorders. Moreover, proapoptotic dependence peptides derived from negative signaling polypeptides are advantageous in that they can be used for the identification. . .
. . .
be performed using methods well known to those skilled in the art. Such methods include, for example, affinity and immunoaffinity selection using ligands, antibodies and anti-idiotype antibodies, for example.
. . .
known to those
skilled in the art. For example, changes in conformation can be assessed by, for example, determining the binding of conformation-specific antibodies or other binding probes, construction and testing of methods known or predicted to influence conformational changes or stability of a polypeptide or by. . .
. . .
nonspecific endocytosis, or through the use of heterologous targeting domain. For example, in a particular embodiment described below, an HIV tat protein, when linked to a dependence peptide, facilitates cellular entry. Lipid carriers also can be used to introduce the nucleic acids encoding proapoptotic dependence peptides, or. . .
. . .
such a heterologous

functional domain that facilitates entry into a cell is the HIV tat protein. This protein or functional equivalents thereof, when coupled to a proapoptotic dependence peptide increases the apoptotic activity of the peptide 30-fold compared to the peptide alone.

. . .

to those skilled in the art. Such domains include, for example, ligands to extracellular proteins or receptors, ligands to other cell surface receptors, antibodies, a natural or engineered viral protein with a 30 desired cell tropism, toxin subunits which facilitate toxin entry and functional fragments thereof.

A heterologous functional domain also can augment the cell death activity of the proapoptotic dependence peptide by linking one or more additional cell death or inhibitory activities onto the proapoptotic dependence peptide. Such cell death or inhibitory activities include, for example,. . . activity. Domains which exhibit apoptotic activity include, for example, ligands or agonists to receptors which induce programmed cell death. Fas ligands or anti-Fas antibodies are two specific examples of such apoptotic domains. A domain which activates caspase protease activity is another example of a heterologous functional domain which exhibits apoptotic activity. Domains which exhibit cytotoxic or cytostatic activity include, for example, toxins and chemotherapeutic agents such as doxorubicin, methotrexate, vincristine and cyclophosphamide can be conjugated to a dependence peptide. Other agents exist as well and are known to those skilled in the art and can be linked to proapoptotic peptides to augment their cell death function.

A heterologous functional domain also can be a regulatable moiety that modulates the activity of a proapoptotic dependence peptide. When linked to a proapoptotic dependence peptide, a modular domain can impart ligand dependent activation or repression of its proapoptotic activity. For example, many different ligand-dependent. . .

. . .

if

hydrophobic, can directly enter cells. Alternatively, in the event that the dependence polypeptide or functional equivalent is intracellular, a test comiound can be

P

conjugated to a targeting moiety, for example, the HIV tat protein, to facilitate cell entry. Incorporation of the test compound into liposomes is. . .

. . .

example, the loss of apoptosis in cells can lead to the pathological accumulation of self-reactive lymphocytes, virally infected cells, hyperproliferative cells such as neoplastic or tumor cells and cells that

contribute to fibrotic conditions. Inappropriate activation of apoptosis also can contribute to a variety of pathological disease states including,. . .

. . .

survival

consisting of cytoplasmically administering a proapoptotic dependence peptide. Further provided is a method of reducing the severity of a pathological

condition consisting of neoplastic, malignant, autoimmune or fibrotic conditions by cytoplasmically administering a proapoptotic dependence peptide.

. . .

resistance to serum proteases additionally can be used as well as other formulations known in the art. For the treatment of a neoplastic or fibrotic condition, the proapoptotic dependence peptide can be administered by direct injection into a solid tumor mass or into a region of fibrosis. Additional modes of administration are known and can be determined by those skilled in the. . .

. . .

vectors containing a natural or engineered envelope protein also can be used to target a nucleic acid encoding a proapoptotic dependence peptide to neoplastic, malignant or autoimmune tissues of cells expressing an appropric-ite cell surface protein. Thus, disorders characterized by cells that abnormally proliferate can be selectively. . .

. . .

The nucleotide sequence of all constructs was confirmed by DNA sequencing. The expression of p751TR protein was detected by flow cytometry using monoclonal antibody 192, and immunoblotting using anti-p75 antiserum (Promega, Madison, WI).

. . .

Acad. Sci. USA 90:4533-4537 (1993)). The expression of p75 ITI protein in the transfected cells was detected by flow cytometry using the monoclonal antibody 192 (Baldwin et al. J.

. . .

FKBP/p75 NTP% protein chimeras containing one or three copies of an FKBP fused to an intracytoplasmic form of p75NTI were expressed in cells. Cross-linking studies indicated that FKBP expressed in cells could be induced to form dimers or multimers by exposing the cells to the FK1012. . .

. . .

of some of the peptides tested. This HIV tat sequence did not affect the function of the peptide to which it was linked, as shown below. For convenience, the hyphen in the above amino acid sequences is a nomenclature intended to set apart the proapoptotic dependence. . .

Peptides which did not exhibit apoptotic activity without the amino-terminal tat sequence similarly did not exhibit apoptotic activity with the linked tat sequence. Specifically, cell viability after exposure to tat-purple was 97.8% for COS-7, 92.8% for PC3 and 69.3% for NTera 2 cells.. . .

To further analyze the effect of particular point mutations on apoptosis, additional studies employing dependence peptides and mutated variants linked to tat were performed in SH-SY5Y cells. The results shown in Figure 2 are of studies in which quadruplicate samples were averaged, and. . .

. . .

and peptide was added to the mitochondria at a final concentration of 385 uM. Western blot analysis using a cytochrome c specific antibody

monitored the amount of cytochrome c released (Ellerby et al. J. Neurosci.- 17:6165-6178 (1997)).

. . .
were washed and resuspended in CFS (50-100 mg/ml) and the final peptide concentration was 385 yM. Western blot analyses using the caspase-3 specific antibody, CPP32, was performed as described (Ellerby et al. J. Neurosci. 17:6165-6178 (1997)).

CLMEN. . . pure proapoptotic dependence peptide comprising substantially the sequence of an active dependence domain selected from the group of 5 dependence polypeptides consisting of p75', androgen receptor, DCC, huntingtin polypeptide, Machado-Joseph disease gene product, SCA1, SCA2. SCA6 and atrophin-1 polypeptide.

2 The proapoptotic dependence peptide of claim 1, wherein the dependence polypeptide is p75 IT' and the proapoptotic dependence peptide further comprises substantially the sequence selected from the group consisting of SATLDALLAALRRI (SEQ ID NO:3), SATLDALLAALGGI (SEQ ID. . .

19 The method of claim 18, wherein said proapoptotic dependence domain-mediated apoptosis is induced by unliganded p75'.

29 The method of claim 28, wherein said pathological condition comprises neoplastic, malignant, autcimmune or fibrotic conditions.

=> d kwic 3

L45 ANSWER 3 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN
ABEN . . . salts thereof, methods of production, intermediates in their production, pharmaceutical compositions containing said compounds, and methods for treating neurodegenerative disorders, tumors of neuronal origin, inflammation, allergy, and pain, and methods for screening compounds that interact with the neurotrophic receptors using said. . .

DETD . . . ART
Nerve growth factor (NGF) was first described by Levi-Montalcini (j, EXp, Zool., 116:321-362 (1951)) as an activity secreted by a mouse sarcoma tumor implanted into a chick embryo. Both sensory ganglion and sympathetic ganglion neurons grew neurites into the sarcoma, which also supported the growth of. . .

. . .
(Kaplan, et al,, Science, 252:554-558 (1991)). More specifically, NGF prevents the development of small-fiber sensory neuropathies that result from the use of taxol, a chemotherapeutic agent (Apfel, et al., Ann. Neurol., 29:87-90 (1991)). NGF is also efficacious against the development of large-fiber sensory neuropathies resulting from the. . .

Effects of NGF on Neuronal Tumors
The importance of NGF in the formation of neuronal tumors has not been firmly established. Certain

investigators believe that excessive synthesis of
is peptide growth factors or their receptors may lead to
the transformation of the recipient cells
(Levi-Montalcini, Science, 237:1154-1162 (1987)). In
fact, it has been shown that brain tumors secrete a
variety of growth factors, including NGF (Westermann,
et al,, J, Neurochem., 50:1747-1758 (1988)), BDNF
(Lichtor, et al., Mol. Cell Neurosci., 2:168-171
(1991)). . .

. . .

Res. Pract., 185:332-338 (1989)), This suggests that
agents that block the effects of NGF may be beneficial
in the treatment of neuronal tumors,
On the other hand, NGF has been utilized as a
reverse transforming agent to halt the progression of
animal tumors of neurodegenic origin (Yaeger, et al,,
Acta. Neuropathol., 83:624-629 (1992)).

. . .

exists evidence to suggest that both
blockade and stimulation of the effects of NGF may be
beneficial for controlling the abnormal growth of
tumors of neuronal origin. Any type of agent that
modulates the interaction between NGF and its receptors
may be considered as a potential. . .

. . .

dependent on NGF for survival, both
in vitro and in vivo. For example, neutralization of
NGF activity in newborn animals by administration of
antibodies against NGF interferes with the normal
development of sensory and sympathetic neurons (Rohrer,
et al,, Development, 103:545-552 (1988)), An important
recent finding is. . . innervating
the point of blockade, and may be a viable option for
pain control in certain patients, such as those with
terminal stage cancer or those undergoing limb removal.

. . .

through
molecular biology techniques to express exclusively
1 receptor or the other, these cell lines (e.g., PC12
or NIH-M) are usually derived from tumors and
is receptor expression levels tend to be much higher than
in native neurons. A method that would allow the study
of the. . .

using 1,11-carbonyldiimidazole (8) as a coupling agent.

. . .

the PNS, such as drug-induced peripheral
neuropathies and the like. The compounds are also
useful as active agents in the treatment of tumors of
neuronal origin, in the treatment of allergy and
inflammation, as analgesic agents, as tools in the
screening for agents with neurotrophic and
antineurotrophic. . .

CLMEN 32 A method for treating tumors of neuronal origin in
mammals which comprises administering an antitumor
effective amount of a compound of the formula
0
N']N
R1
N
1
X2
or a salt thereof, wherein

SUBSTITUTE. . .
. . .
compound being screened with a predetermined amount of radiolabeled neurotrophic factor in the presence of a predetermined amount of cells that express both p75 and a trk receptor and a predetermined amount of a compound of the formula
0
ANvoN
(al R1
N
h2
or a salt thereof, in a pharmaceutical. . .

32 A method for treating tumors of neuronal orgin in mammals which comprises administering an antitumor effective amount of a compound of the formula
0
NopoN
R1
N
I
A2
or a salt thereof, wherein
AMENDED. . .

=> d kwic 1

L45 ANSWER 1 OF 3 PCTFULL COPYRIGHT 2007 Univentio on STN
TIEN HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10
TIFR TR10, RECEPTEUR DE FACTEUR DE NECROSE TUMORALE HUMAIN
ABEN The present invention relates to a novel protein, TR10, which is a member of the tumor necrosis factor (TNF) receptor superfamily and the TRAIL receptor subfamily. In particular, isolated nucleic acid molecules are provided encoding the. . .
ABFR . . . invention concerne une nouvelle proteine, la TR10, qui constitue un membre de la superfamille des recepteurs de facteur de necrose tumorale (TNF) et de la sous-famille de recepteur TRAIL . En particulier, cette invention concerne des molecules d'acide nucleique isolees codant. . .

DETD HUMAN TUMOR NECROSIS FACTOR RECEPTOR TR10
FIELD OF THE INVENTION
The present invention relates to a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encodiner a novel human tumor necrosis factor receptor, TRIO. TRIO polypeptides are also provided, as are vectors, host cells, and recombinant methods for producing the same, and antibodies that bind to TRIO polypeptides. The invention further relates to screening methods for identifying rn agonists and antagonists of TRIO activity.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines, which act through TNF receptors to regulate numerous biological processes, including protection against infection. . .

. . .
(R. Watanabe-Fukunaga et al., Nature 356:314
(1992)), perhaps reflecting a failure of programmed cell death.
Mutations of the CD40 ligand
cause an X-linked immunodeficiency state characterized by high
levels of immunoglobulin M
and low levels of immunoolobulin G in plasma, indicating faulty
T-cell-dependent B-cell
activation. . .
. . .
number of biological effects elicited by TNF and LT-, acting through
their
LI In Z_ Z__
receptors, include hemorrhacric necrosis of transplanted tumors
, cytotoxicity, a role in
endotoxic shock, inflammation, immunoregulation, proliferation and
anti-viral responses, as
well as protection against the deleterious effects of ionizing. . .
radiation. TNF and LT- are
involved in the pathogenesis of a wide rancre of diseases, including
endotoxic shock, cerebral
1_] Zn tn
malaria, tumors, autolmmune disease, AIDS and graft-host
rejection (B. Beutler and C. Von
Huffel, Science 264:667-668 (1994)). Mutations in the p55 receptor
cause. . .
. . .
and homeostasis of multicellular organisms (H. Steller, Science
267:1445-1449
(1995)). Derangements of apoptosis contribute to the pathogenesis of
several human diseases
including cancer, neurodegenerative disorders, and acquired
immune deficiency syndrome
(C.B. Thompson, Science 267:1456-1462 (1995)). Recently, much attention
has focused on
the signal transduction and. . .
. . .
detecting over-expression of TRIO, or soluble form
thereof, compared to normal control tissue samples may be used to detect
the presence of
tumors.

Tumor Necrosis Factor (TNF) family ligands are known to be
amoner the most
plelotropic cytokines, inducing a larcre number of cellular responses,.
. .
. . .
dysregulation can lead to a number of
different pathogenic processes. Diseases associated with increased cell
survival, or the
inhibition of apoptosis, include cancers, autolmmune
disorders, viral infections, inflammation,
graft vs. host disease, acute graft rejection, and chronic graft
rejection. Diseases associated
with increased apoptosis include. . .
. . .
following
tissues: fetal liver, peripheral blood lymphocytes (PBL), lung, kidney,
small intestine, colon,
endothelial cells, and monocyte activated tissue. Furthermore, the
following cancer cell lines
express TRIO: Hela cell S3, SW480 (colorectal adenocarcinoma), and A549
(lung carcinoma).
. . .

prostate, testis, ovary, small intestine, colon, PBLs, lymph node, bone marrow and
fetal liver. TRIO expression was not observed in most cancer cell lines tested. See Example 7,
below.

. . .
Zn LI
(e.g., inhibit) hematopolesis), anti crenicity (ability to bind (or compete with a TRIO polypeptide
for binding) to an anti-TR IO antibody), immunogenicity (ability to generate antibody which
binds to a TRIO polypeptide), ability to form multimers with TRIO polypeptides of the
invention, and ability to bind to a. . .

. . .
one embodiment where one is assaying for the ability to bind or compete with full-length TRIO polypeptide for binding to anti-TRIO antibody, various
immunoassays known in the art can be used, includincr but not limited to, competitive and non-
competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme
linked immunosorbent assay), sandwich immunoassays, immunoradiometric assays, gel
diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using
colloidal gold, enzyme or radioisotope labels',. . . C, C] C] LI ' I I
complement fixation assays, immunofluorescence assays, protein A assays, and
immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by
detectino a label on the primary antibody. In another embodiment, the primary antibody is
-i detected by detecting binding of a secondary antibody or rea(yent to the primary antibody. In a
further embodiment, the secondary antibody is labeled. Many means are known in the art for
detectiner bindina in an immunoassay and are within the scope of. . .

. . .
a disease which results from under-expression over-expression or altered expression of TRIO or a soluble form thereof, such as,
for example, tumors or autoimmune disease.

. . .
of
polynucleotides and polypeptides of the present invention. Generally, such vectors comprise
cis-actincy control recylons effective for expression in a host operatively linked to the
polynucleotide to be expressed. Appropriate trans-actincr factors either are supplied by the
In
host, supplied by a complementina vector or supplied. . .

The DNA insert should be operatively linked to an appropriate promoter, such as the
phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40. . .

. . .
applications, particularly those that make use of the chemical and biological properties of TRIO. Among these are applications in treatment of tumors,
resistance to parasites, bacteria and viruses, to induce proliferation

of T-cells,' endothelial cells
and certain hematopoletic cells, to treat restenosis araft vs.. . . .
. . .
the animal, in situ hybridization
analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transcrenic gene-expressing
tissue may also be evaluated immunocytochemically or
Immunohistochemically usinCF
in
antibodies specific for the transgene product.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or
covalent associations and/or may be indirectly linked, by for example, liposome formation.
. . .
In
another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or
heterotetramers, are fon-ned when proteins of the invention contact antibodies to the
polypeptides of the invention (including antibodies to the heterolocrous polypeptide sequence in
t) zn
a fusion protein of the invention) in solution. In other embodiments, multimers of the
invention. . . recited in SEQ ID
In
2o NO:2 or the polypeptide encoded by the deposited cDNA clone). In one instance, the covalent
associations are cross-linking between cysteine residues located within the polypeptide
sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In
another. . . are herein incorporated by reference in its entirety). In another embodiment, two or more TRIO polypeptides of the invention are
through synthetic linkers (e.cr., peptide, carbohydrate or soluble polymer linkers). Examples
include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by
reference). Proteins comprising multiple TRIO polypeptides separated by peptide linkers may
be produced using conventional recombinant DNA technology.
. . .
(each of which is hereby incorporated by reference). In further preferred
embodiments, a TRIO-FLAG fusion protein is detectable by anti-FLAG monoclonal antibodies (also
available from Slorma).
. . .
chemical techniques known in
the art. For example, proteins desired to be contained in the multimers of the invention may be
chemically cross-linked using linker molecules and
linker molecule length optimization
techniques known in the art (see, e.cy., US Patent Number 5,478,925, which is herein
incorporated by reference in its. . . entirety). Additionally, multimers of the invention may be
generated using techniques known in the art to form one or more inter-molecule cross-links
between the cysteine residues located within the polypeptide sequence of the proteins desired to

be contained in the multimer (see, e.cr., US. . .
. . .
the invention are generated by ligating a polynucleotide sequence encoding a
C] In
polypeptide of the invention to a sequence encodina a linker polypeptide and then further to a
synthetic polynucleotide encoding the translated product of the polypeptide in the reverse
orientation from the oricrinal. . .
. . .
to bind TRIO
L] LI
licrand) may still be retained. For example, the ability of shortened TRIO rnuteins to induce
tn
and/or bind to antibodies which recognize the complete or mature forms of the polypeptides
C
generally will be retained when less than the majority of the. . .
. . .
and/or endothelial cells) may still be retained. For example the ability of the shortened TRIO mutein to induce and/or bind to antibodies which
recounize the complete or mature forms of the polypeptide generally will be retained when less
than the majority of the residues. . .
. . .
cells chosen. For example, cysteme residues can be deleted or substituted with another amino acid residue in order to eliminate
disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for
Ln t_1
example, expression of a hornocreneous product that is more easily recovered and purified from
yeast hosts which are known to hypererlycosylate N-linked sites. To this end, a variety of
amino acid substitutions at one or both of the first or third amino acid. . .
. . .
which are differentially
modified durina or after translation, e.cr., by glycosylation, acetylation, phosphorylation,
Z.- Zn
amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to
L_ in tn in
'body molecule or other cellular licrand, etc. Any of numerous chemical modifications
an anti t]
may be carried out. . .

Additional post-translational modifications encompassed by the invention include, for
example, e.cr., N-linked or 0-linked carbohydrate chains, processing of N-terminal or
C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical
modifications of N-linked or 0-linked carbohydrate chains, and addition or deletion of an
N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides
may. . .

. . .
a number of attachment methods available to those skilled in the art, e.g., EP 0 401
384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp.

Zr
As sucrorested above, polyethylene glycol may be attached to proteins via linkage to any
In r-I zn L]
of a number of amino acid residues. For example, polyethylene erlycol can be linked to a
proteins via covalent bonds to lysine, histidine, aspartic acid, alutamic acid, or cysteine
Z--
residues. One or more reaction chemistries may. . .
. . .
accomplished by
any number of means. For example, polyethylene glycol may be attached to the protein either
directly or by an intervening linker. Linkerless
systems for attaching polyethylene erlycol to
z1_ Z1- C]
proteins are described in Del cyado et al., Crit. Rev. Thera. Drug Carrier. . .

One system for attaching polyethylene glycol directly to amino acid residues of proteins
t- tn
without an intervening linker employs tresylated MPEG, which is produced by the modification
of monmethoxy polyethylene glycol (MPEG) using tresylchlorlde (ClSO2CH2CF,). Upon
reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine
groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates
produced by reacting proteins of the invention with a polyethylene (Flycol molecule having a
tn
2,2,2-trifluoreothane sulphonyl group.

Polyethylene glycol can also be attached to proteins usiner a number of different
zn zn
intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which
is incorporated herein by reference, discloses urethane linkers for connecting polyethylene
glycol to proteins. Protein-polyethylene crlycol conjugates wherein the polyethylene glycol is
attached to the protein by a linker can also be produced by reaction of proteins with compounds
such as MPEG-succinimidyisuccinate, MPEG activated with 1, I'-carbonyidlimidazole,
MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitroph&noicarbonate, and various. . .
. . .
protein of the invention
(i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the
invention may be linked, on average, to 1, 2, 31 4, 5] 69 71
8, 91 10, 12, 15, 17, 20, or more
polyethylene glycol. . .

. . .
heme moiety, covalent attachment of a nucleotide or
nucleotide derivative, covalent attachment of a lipid or lipid
denivative, covalent attachment of
phosphotidylinositol, cross-linkiner, cyclization, dlsulfide
bond formation, demethylation,
formation of covalent cross-links, formation of cysteine,
formation of pyroglutamate,
formylation, gamma-carboxylation, glycosylation, GPI anchor formation,
hydroxylation,
In
iodination, methylation, myristoylation, oxidation, peaylation,
proteolytic processing
tn zn I
phosphorylation,. . .
. . .
the polynucleotide encoding this
polypeptide. An Immunocrenic epitope, as used herein, is defined as a
portion of a
protein that elicits an antibody response in an animal, as
determined by any method
known in the art, for example, by the methods for creneratincr
antibodies described
infra. (See, for example, Geysen et al., Proc. Nati. Acad. Sci. USA
81:3998-4002
(1983)). The term antigenic epitope, as used herein, is defined as a
portion of a
r_1
protein to which an antibody can immunospecifically bind its
antigen as determined by
Zn
any method well known in the art, for example, by the immunoassays
described
herein.. . .
. . .
85, 90, 95, or
100 amino acid residues in lencrth. Antigenic epitopes are useful, for
example, to raise
tn L_
'bodies, includinu monoclonal antibodies, that specifically
bind the epitope.

Non-11miting examples of antigenic polypeptides or peptides that can be
used to
L]
generate TRIO receptor-specific antibodies include: a
polypeptide comprising amino acid
'dues from about 57 to about 113 Figures I A-D (2 to 58 in SEQ. . . .
. . .
et al., J. Gen. Virol. 66:2347-2354 (1985). The polypeptides comprising
one or more immunogenic epitopes may be presented for eliciting an
antibody response
together with a carrier protein, such as an albumin, to an animal system
(such as, for
example, rabbit or mouse), or,. . . a carrier. However,
immunogenic epitopes comprising as few as 8 to 10 amino acids have been
shown to
be sufficient to raise antibodies capable of binding to, at
the very least, linear epitopes
in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to
induce
antibodies according to methods well known in the art

including, but not limited to, in
:_n Z__
vivo immunization, in vitro immunization, and. . . al., J. Gen. Virol., 66:2347-
2354 (1985). If in vivo immunization is used, animals may be immunized with free
peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to
a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid.

For instance, peptides containing cysteine residues may be coupled to a carrier using a
linker such as maleimidobenzoyi-N-hydroxysuccinimide ester (MBS), while other
peptides may be coupled to carriers using a more general linking agent such as
Zn in Z17
glutaraldehyde. Animals such as, for example, rabbits, rats, and mice are immunized
with either free or carrier-coupled peptides, for instance, by intrapentoneal and/or
intradermal injection of emulsions containing about 100 micrograms of peptide or
C!
carrier protein and Freund's adjuvant. . . Several booster injections may be needed, for instance, at intervals
of about two weeks, to provide a useful titer of anti-peptide antibody that can be
detected, for example, by ELISA assay using free peptide adsorbed to a solid surface.

The titer of anti-peptide antibodies in serum from an immunized animal may be
increased by selection of anti-peptide antibodies, for instance, by adsorption to the
peptide on a solid support and elution of the selected antibodies according to methods
well known in the art.

. . .
chains of mammalian immunoolobulins. See, e.cr., EP
t] 1-In tn
394,827; Traunecker et al., Nature, 331:84-86 (1988). IgG Fusion proteins that have
a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also
been found to be more efficient in binding and neutralizing. . .
. . .
detecting over-expression of TRIO, or
soluble form thereof, compared to normal control tissue samples may be used to detect the
presence of tumors, for example. Assay techniques that can be used to determine levels of a
protein, such as a TRIO protein of the. . .

Preferred for assaying TRIO protein levels in a biological sample are antibody-based
techniques. For example, TRIO protein expression in tissues can be studied with classical
immunohistological methods. (M. Jalkanen et al., J. Cell. Biol.. . .

Jalkanen et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful
for detectincy TRIO receptor gene expression include immunoassays, such

as the enzyme linked
Z] Zn
immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable antibody assay labels are known in the art and
include enzyme labels, such as,
glucose oxidase; radioisotopes, such as iodine ('I, 125i,. . .

Antibodies
The present invention further relates to antibodies and T-cell
antigen receptors
(TCR) which immunospecifically bind a polypeptide, preferably an
epitope, of the
present invention (as determined by immunoassays well known in the art
for assaying
specific antibody-antigen binding). Antibodies of
the invention include, but are not
limited to, polyclonal, monoclonal, multispecific, human, humanized or
chimeric
'bodies, single chain antibodies, Fab fragments, F(ab')
fragments, fragments
anti I rn tn
produced by a Fab expression library, anti-idiotypic (anti-Id)
antibodies (including
e.g., anti-Id antibodies to antibodies of the
invention), and epitope-binding fragments
of any of the above. The term antibody, as used herein, refers
to immunoglobulin
molecules and immunologically active portions of immunoglobulin
molecules, i.e.,
molecules that contain an anticren binding site. . .

Most preferably the antibodies are human anti gen-binding
antibody fragments
n t) in
of the present invention and include, but are not limited to, Fab,
FaVand F(ab')2, Fd,
single-chain Fvs (scFv), single-chain antibodies, disulfide-
linked Fvs (sdFv) and
fragments comprising either a VL or VH domain. Antigen-binding
antibody
tn
fracyments, Includincy sincyle-cham antibodies, may comprise
the variable region(s)
Z] Z1_ L]
alone or in combination with the entirety or a portion of the followina:
hinue. . . In
fragments also comprising any combination of variable recrion(s) with a
hincre region,
Z]1 L] L_ in
CHI, CH2, and CH3 domains. The antibodies of the invention may
be from any
animal orialn includincr birds and mammals. Preferably, the
antibodies are human,
murine, donkey, ship rabbit, croat, cruinea pia, camel, horse, or
chicken. As used
LI ;_n Z]
herein, human antibodies include antibodies having
the amino acid sequence of a
human immunoorlobulin and include antibodies isolated from
human immunoorlobulin
C 4n
libraries or from animals transgenic for one or more human
immunoulobulin and that

do not express endocrenous. . .

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of. . .

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the. . . herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies that specifically bind any epitope or polypeptide of the present invention may also be excluded.

Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%,. . . known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less. . .

Further included in the present invention are antibodies that bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention.. . .

The invention also provides antibodies that competitively inhibit bindiner of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example,. . .

In
In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at. . .

Antibodies of the present invention may act as acyonists or antagonists of the
C tn
polypeptides of the present invention. For example, the present

invention includes
antibodies which disrupt the receptor/ligand interactions with the polypeptides of the
invention either partially or fully. The invention features both receptor-specific
antibodies and ligand-specific antibodies. The
invention also features receptor-specific
antibodies which do not prevent ligand binding but prevent receptor activation.

serine/threonine) of the receptor or its
substrate by immunoprecipitation followed by western blot analysis (for example, as
described supra). In specific embodiments, antibodies are provided that inhibit ligand
or receptor activity by at least 90%, at least 80%, at least 70%, at least 60%, or at least
50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent
ligand binding and receptor activation as well as antibodies that recognize the receptor-
figand complex, and, preferably, do not specifically recognize the unbound receptor or
the unbound ligand. Likewise, included in the invention are neutralizino antibodies
which bind the ligand and prevent binding of the herand to the receptor, as well as
L_ Z7 t]
'bodies which bind the. . . I
prevent the licrand from binding the receptor. Further the
L] n I I I ion are
'bodies which activate the receptor. These antibodies may act as receptor agorusts,
anti I I I
1 e., potentiate or activate e ther all or a subset of the biological activities of the I'cyand
mediated receptor activation. The antibodies may be specified as agonists, antagonists
or inverse agonists for biological activities comprising the specific biological activities
4n t-I LI LI
of the peptides of the invention disclosed herein. The above antibody agoilists can be
made using methods known in the art. See, e.g., PCT publication WO 96/40281;
U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen, et al.,
Cancer Res. 58(16):3668-3678 (1998)-l Harrop et al., J. Immunol. 161(4):1786-1794
(1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon, et al., J. Immunol.

Antibodies of the present invention may be used, for example, but not limited
to, to purify, detect, and target the polypeptides of the present invention, including
both in vitro and in vivo diagnostic and therapeutic methods. For example, the
antibodies have use in immunoassays for qualitatively and quantitatively measuring
levels of the polypeptides of the present invention in biological samples. See, e.g

z-- tn
Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory
Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may
be used either alone or in combination with other compositions. The antibodies may
further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus
or chemically conjugated (including covalently and non-covalently conjugations) to
polypeptides or other compositions. For example, antibodies of the present invention
may be recombinantly fused or conjugated to molecules useful as labels in detection
assays and effector molecules such as heterologous poiypeptides, drugs, or toxins.

The antibodies of the invention include derivatives that are modified, i.e, by the
covalent attachment of any type of molecule to the antibody such that covalent
attachment does not prevent the antibody from generating an anti-idiotypic response.

n tr
For example, but not by way of limitation, the antibody derivatives include antibodies
that have been modified, e.o., by alycosylation, acetylation, peoylation,
phosphylation, arrildation, derivatization by known protecting/blockincy groups,
proteolytic cleavage, linkage to a cellular 11,.gand or other protein, etc. Any of
numerous chemical modifications may be carried out by known techniques, including
but. . .

The antibodies of the present invention may be crenerated by any suitable
method known in the art. Polyclonal antibodies to an antigen-of- interest can be
produced by various procedures well known in the art. For example, a polypeptide of
the invention. . . administered to various host animals includincy, but not limited to,
rabbits, mice, rats, etc. to induce the production of sera containing polyclonal
antibodies specific for the antigen. Various adjuvants may be used to increase the
immunolocrical response, depending on the host species, and include. .
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Monoclonal antibodies can be prepared using a wide variety of techniques
In
known in the art including the use of hybridoma, recombinant, and phage display
L_
technologies, or a combination thereof. For example, monoclonal antibodies can be
In

produced using hybridorna techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Harnmerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term monoclonal antibody as used herein is not limited to antibodies produced through hybridorna technology. The term monoclonal tn antibody refers to an antibody that is derived from a single clone, including any t]) eukaryotic, prokaryotic, or phage clone, and not the method by which it. . .

]n Methods for producing and screening for specific antibodies using hybridoma tn technology are routine and well-known in the art and are discussed in detail in Example In 6, below. Briefly, mice can. . . be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.cr., antibodies r] In specific for the antigen are detected in the mouse serum, the mouse spleen is harvested In and splenocytes isolated. The splenocytes are. . . and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains hlcyh levels of antibodies, can be (zenerated by immunizing mice with positive hybridorna clones.

Accordingly, the present invention provides methods of generating monoclonal C] Z74 n antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridorna is crenerated by fusing splenocytes isolated from a Mouse immunized with an antigen of the invention with myelorna cells and then screening the hybridomas resultine from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments that recognize specific epitopes may be generated by Z.- Zn known techniques. For example, Fab and F(ab')2 fragments of the invention. . .

For example, the antibodies of the present invention can also be cyenerated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phacre can be I In utilized to display antigen-binding domains expressed from a repertoire or L] combinatorial antibody library (e.g., human or murine). Phage expressing an antigen t] C] L- binding domain that binds the antigen of interest can be. . . these methods are typically filamentous phage including fd and Z- M13 bindincr domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage crene III or gene VIII protein. Examples of phacre display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol.

. Queen et al., U.S. Patent No. 5,585,089-, Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-(Yraftinc, (EP 239,400; PCT publication WO 91/09967;. . .

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoolobulin sequences. See also, U.S. Patent Nos.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoolobulins, but which can express human immunoglobulin crenes.. . . or simultaneously with the introduction of human immunoglobulin loci by homolocyous C Zr recombination. In particular, homozygous deletion of the JH region prevents endooenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The Zn r_1 transgenic mice are immunized in the normal fashion with a selected anticren, e.g., all

or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transcrenic mice using conventional hybridorna technology. The human immunocriobulin transgenes harbored by. . . and somatic mutation. Thus, usiner such a technique, it is possible to producetherapeuticallyusefulloG,IaA.lerMandicrEantibodies. Foranoverviewof t7] ZI) LI C this technology for producing human antibodies. see Lonberg and HLlszar (1995, Int.

Z__ Z7] Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.(Y., PCT publications WO 98/24893; WO 96/34096; WO 96/33735; U.S. Patent Nos. 5,413,923; 5,625,126-1 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; and 5,939,598,. . . entirety. In addition, companies such as Abcyenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

L_ L] Zn Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as guided selection. In this approach a selected non-human monoclonal antibody, e.a., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et tn al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idlotype antibodies that mimic polypeptides of the invention using 4n techniques well known to those skilled in the art. (See, e.cy., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be. . .

r-1 Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a poiypeptide

of the invention,
preferably, an antibody that binds to a polypeptide having the amino acid sequence of
SEQ ID NO:2.
. . .
the nucleotide sequence of the
polynucleotides determined, by any method known in the art. For example, if the
nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody
may be assembled from chemically synthesized olloonucleotides (e.cr., as described in
Kutmeler et al., BloTechniques 17:242 (1994)), which, briefly, involves the synthesis
of. . .

In
Alternatively, a polynucleotide encoding an antibody may be generated from
nucleic acid from a suitable source. If a clone containina a nucleic acid encodina a
r-1 r_1
particular antibody is not available, but the sequence of the antibody molecule is
known, a nucleic acid encodiner the immunocriobulin may be obtained from a suitable
I= Z:]
source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic
preferably poly A+ RNA, isolated from, any tissue or cells expressing the
acl
antibody, such as hybridoma cells selected to express an antibody of the invention) by
PCR amplification using synthetic primers hybridizable to the Yand 5'ends of the
sequence or by cloning using an. . . oligonucleotide probe specific for the particular gene
n Zn
sequence to identify, e.a., a cDNA clone from a cDNA library that encodes the
antibody. Amplified nucleic acids generated by PCR may then be cloned into
replicable cloning vectors using any method well known in the. . .

Once the nucleotide sequence and corresponding amino acid sequence of the
antibody is determined, the nucleotide sequence of the antibody may be manipulated
using methods well known in the art for the manipulation of nucleotide sequences,
e.g., recombinant DNA techniques, site directed. . . Protocols in Molecular Biology, John
Wiley & Sons, NY, which are both incorporated by reference herein in their entireties
), to generate antibodies having a different amino acid sequence, for example to create
amino acid substitutions, deletions, and/or insertions.
. . .
for a listing of human framework
regions). Preferably, the polynucleotide crenerated by the combination of the
framework regions and CDRs encodes an antibody that specifically binds a

polypeptide of the invention. Preferably, as discussed supra, one or more amino acid
substitutions may be made within the framework recrions, and, preferably, the amino
acid substitutions improve binding of the antibody to its antiaen. Additionally, such
In t:)
methods may be used to make amino acid substitutions or deletions of one or more
variable recrion cystelne residues participating in an intrachain disulfide bond to
tn LI
,crenerate antibody molecules lacking one or more intrachain disulfide bonds. Other
alterations to the polynucleotide are encompassed by the present invention and within
the. . .

In addition, techniques developed for the production of chimeric antibodies
(Morrison et al., 1984, Proc. Nati. Acad. Scl. 81:851-855; Neuberger et al., 1984,
Zn
Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from
a mouse antibody molecule of appropriate antigen specificity together with genes from
a human antibody molecule of appropriate biological activity can be used. As
described supra, a chimeric antibody is a molecule in which different portions are
derived from different animal species, such as those having a variable recrion derived
from a murine mAb and a human immurioglobulin constant recrion, e.g., humanized
antibodies.

Alternatively, techniques described for the production of single chain Z-)
antibodies (U.S. Patent No. 4,694,778; Bird, 1988, Science 242:423- 42; Huston et
al., 1988, Proc. Nati. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature
334:544-54) can be adapted to produce single chain antibodies. Single chain
n tn
antibodies are formed by linking the heavy and light chain fragments of the Fv region
Z:1 L]
via an amino acid bridge, resulting in a single chain. . .

Methods of Producing Antibodies
The antibodies of the invention can be produced by any method known in the
art for the synthesis of antibodies, in particular, by chemical synthesis or preferably,
by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment,
derivative or analocr thereof, e.g., a heavy or light chain of an antibody of the
invention, requires construction of an expression vector containina a polynucleotide

that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a In ?_1 C] protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences r-1 Z-1 and appropriate transcriptional and translational control siGnals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PC7 Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

. . .

is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule,. . .

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria

(e.g., E. coli, B. subtills) transformed with recombinant bacteriophage DNA, plasmid
DNA or cosmid DNA expression vectors containing antibody codineF sequences; yeast
(e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors
containincy antibody coding sequences; insect cell systems infected with recombinant
virus expression vectors (e.cy., baculovirus) containing antibody coding sequences,
r-1 Z-- in
plant cell systems infected with recombinant virus expression vectors (e.g-,
cauliflower mosaic virus, CaNIV; tobacco mosaic virus, TMV) or transformed with
recombinant plasmid expression vectors (e.cy., Ti plasmid) containing antibody coding
t] zn
sequences; or mammalian cell systems (e.u., COS, CHO, BHK, 293, 3T3 cells)
Z1-
harborincr recombinant expression constructs containing promoters derived from. . .

In
Preferably, bacterial cells such as Escherichia coil, and more preferably, eukaryotic
cells, especially for the expression of whole recombinant antibody molecule, are used
for the expression of a recombinant antibody molecule. For example, mammalian
cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as
the major intermediate. . .

In bacterial systems, a number of expression vectors may be advantageously
selected depending upon the use intended for the antibody molecule being expressed.
. .
example, when a larcre quantity of such a protein is to be produced, for the
In
generation of pharmaceutical compositions of an antibody molecule, vectors which
direct the expression of hi cyh levels of fusion protein products that are readily purified
Zn
may be desirable. Such. . . but are not limited, to the E. coli expression
vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding
sequence may be ligated individually into the vector in frame with the lac Z coding
region so that a fusion protein. . .
. .
californica nuclear polyhedrosts virus
tn
(AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frucylperda cells. The antibody coding sequence may be cloned
Zn Z.-
individually into non-essential regions (for example the polyhedrin gene) of the virus

and placed under control. . .

. . .

a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation io control complex, e.cr., the late promoter and tripartite leader sequence.. . . (e.cr., region tn Zn cl Zn El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.cr., see Logan & Shenk, 1984, Proc. Nati.

Z Z
Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the tn ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the. . .

. . .

host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T35 W138, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HT132, BT20 and T47D, and normal mammary gland cell zn line such as, for example,. . .

For loner-term, hi crh-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be encrineered. Rather than using expression vectors which contain viral oricrins of replication, host cells can be transformed with DNA. . .

. . .

can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule.

. . .

Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification. . . cloned genes in mammalian cells in DNA cloning, Vol (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker Crene. Since the amplified recrion is associated with the antibody crene, production of the antibody will t] In also increase (Crouse et al., 1983, Mol. Cell. Blol. 3:257).

cl 9
Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification. . .

Antibody Cojugates

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention to crenerate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20 or 50 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the Z' polypeptides of the present Invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in. . .

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable realons. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CHI domain, CH2 domain, and CH3 domain or any t] tn combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding. . . Fc: portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IorA and IcFM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT. . .

Moreover, the antibodies or fragments thereof of the present invention can be

fused to marker sequences, such as a peptide to facilitates their purification.. . .

. . .

derived from the influenza hernagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the flag tag

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The present invention further encompasses antibodies or fragments thereof

conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.a., determine the efficacy of a given treatment

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regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting. . .

Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of. . .

Further, an antibody or fragment thereof may be conjugated to a therapeutic

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moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or

In

a radioactive metal ion.. . . acrent that is

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detrimental to cells. Examples include paclitaxol, cytochalasin B, Orramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, crlucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

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(BSNU) and lomustine (CCNU), cyclothospharnide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (11) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.cy., vincristine and vinblastine).

. . .

Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon,

B-interferon, nerve growth
factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent
or an anti- angiogenic aorent,. . .

Antibodies may also be attached to solid supports. which are particularly useful
I I
for immunoassays or purification of the tarcret anti(yen. Such. . .

Techniques for conjugating such therapeutic moiety to antibodies are well
known, see, e.cr., Arnon et al., Monoclonal Antibodies For Immunotargeting Of
L_ t! Zn
Drucrs In Cancer Therapy, in Monoclonal Antibodies And Cancer Therapy, Relsfeld
L]
et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., Antibodies For
Drua Delivery, in Controlled Drucr Delivery (2nd Ed.), Robinson et al. (eds.), pp.

In Zr
623-53 (Marcel Dekker, Inc. 1987); Thorpe, Antibody Carriers Of Cytotoxic Agents
Zn
In Cancer Therapy: A Review, in Monoclonal Antibodies '84: Biological And Clinical
Applications, Pinchera et al. (eds.), pp. 475-506 (1985); Analysis, Results, And
Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer
Therapy, in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et
al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., The Preparation
And Cytotoxic Properties Of Antibody-Toxin Conjugates , Immunol. Rev. 62:119-58
(1982).

Alternatively, an antibody can be conjugated to a second antibody to form an
g I
antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which
is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it,
administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can
be used as a therapeutic.

Assays For Antibody Binding
The antibodies of the invention may be assayed for immunospecific binding by
any method known in the art. The immunoassays which can be used include but are
not limited to competitive and non-competitive assay systems usiner techniques such as
western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay),
sandwich immunoassays, immunoprecipitation assays, precipitin reactions,

gel
diffusion precipitin reactions, immunodiffusion assays, agglutination assays,
complement-fixation assays, immunoradiometric assays, fluorescent immunoassays,
protein A. . .
. . .
phosphate at pH 7.2, 1 %
Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.gr-,
EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell
lysate, incubating for a period of time (e.cr., 1-4 hours) at 4' C, addina protein A
Z7. . . or
more at 4' C, washing the beads in lysis buffer and resuspending the beads in
SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a
particular antigen can be assessed by, e. a., western blot analysis. One of skill in the
art would be knowledgeable as to the parameters that can be modified to increase the
binding of the antibody to an antigen and decrease the background (e.g., pre-clearing
the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see,. . .
. . .
or non-
In in
fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking
In n Zn C
the membrane with primary antibody (the antibody of interest) diluted in blocking
buffer, washing the membrane in washing buffer, blocking the membrane with a
secondary antibody (which recognizes the primary antibody, e.g., an anti-human
antibody) conjugated to an enzymatic substrate (e.cr., horseradish peroxidase or
alkaline phosphatase) or radioactive molecule (e.g., 32P or 1251) diluted in blocking
buffer, washing. . .

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter
plate with the antigen, adding the antibody of interest conjugated to a detectable
L tn n
compound such as an enzymatic substrate (e.cr., horseradish peroxidase or alkaline
phosphatase) to the well and incubating for a period of time, and detecting the
Z4 LI
presence of the anticren. In ELISAs the antibody of interest does not have to be
conjugated to a detectable compound-, instead, a second antibody (which recognizes
the antibody of interest) conjugated to a detectable compound may be added to the
well. Further, instead of coatina the well with the antigen, the antibody may be coated
to the well. In this case, a second antibody

conjugated to a detectable compound may
be added followina the addition of the antigen of interest to the coated
well. One of
skill. . .
. . .
Current
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Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New
York at
The binding affinity of an antibody to an antigen and the
off-rate of an
In
antibody-antigen interaction can be determined by competitive
binding assays. One
example of a competitive binding assay is a radioimmunoassay comprising
the
incubation of labeled antigen (e.g., 3H or 1251) with the
antibody of interest in the
Z-1 in
presence of increasing amounts of unlabeled antigen, and the detection
of the antibody
Zln
bound to the labeled anticren. The affinity of the antibody of
interest for a particular
C]
antigen and the binding off-rates can be determined from the data by
scatchard plot
analysis. Competition with a second antibody can also be
determined using
In
radioimmunoassays. In this case, the anticren is incubated with
antibody of interest is
conjugated to a labeled compound (e.cr., 3H or 1251) in the
presence of increasing
amounts of an unlabeled second antibody.

Antibody-Based Therapeutic Uses
The present invention is further directed to antibody-based
therapies which
involve administerina antibodies of the invention to an
animal, preferably a mammal,
rn
and most preferably a human, patient for treating one or more of. . .
to,
'bodies of the invention (including fragments, analogs and derivatives
thereof as
anti I I Z' Zn
described herein) and nucleic acids encoding antibodies of the
invention (including
fragments, analogs and derivatives thereof as described herein). The
antibodies of the
invention can be used to treat, inhibit or prevent diseases and
disorders associated
with aberrant expression and/or activity of a. . . and/or activity of
a
polypeptide of the invention includes, but is not limited to,
alleviating symptoms
associated with those diseases and disorders. Antibodies of
the invention may be
ided in pharmaceutically acceptable compositions as known in the art or
as
provi I I 1
described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCQ. Some of these approaches are described in more detail below. Armed with the teachiners provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e. g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fraorrients derivatives, analogs, or nucleic acids, are administered LI 47] to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or C] neutralizing antibodies against polypeptides or polynucleotides of the present In invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fraerments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments :r thereof. Preferred bindincr affinities include those with a. . .

Antibody-Based Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity. . .

In a preferred aspect, the compound comprises nucletc acid sequences encoding an antibody, said nucleic acid sequences beiner part of expression vectors zn zn that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding recrion, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a C in desired site in the genorne, thus providing for intrachromosornal expression of the antibody nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; ZijIstra et al., 1989, Nature 342:435-438). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

. . . or coating with lipids or cell-surface receptors or transfecting zn In agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used. . .

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599).. . . that are not necessary for packaging of the viral crenome and integration into host cell DNA. The nucleic acid sequences rn encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a. . .

In an embodiment in which recombinant cells are used in gene therapy, nucleic Z]- acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are. . .

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked

to the codin CF re Gion,
t] zn
such that expression of the nucleic acid is controllable by controlling the presence or
zn
absence. . .

Demonstration of Antibody-Based Therapeutic or Prophylactic Activity
The compounds or pharmaceutical compositions of the invention are preferably
tested in vitro, and then in vivo for the. . .

Antibody-Based TherapeuticlProphylactic Administration and Composition
The invention provides methods of treatment, inhibition and prophylaxis by
administration to a subject of an effective amount of a compound or pharmaceutical
composition of the invention, preferably an antibody of the invention. In a preferred
aspect, the compound is substantially purified (e.g., substantially free from
substances that limit its effect or. . .

. . .
a porous, non-porous, or gelatinous material,
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includincr membranes, such as sialastic membranes, or fibers. Preferably, when
administering a protein, including an antibody, of the invention, care must be taken to
use materials to which the protein does not absorb.

. . .
in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et
al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestem
and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp.

. . .
aun; Blolistic, Dupont), or coating
tn n C LI
with lipids or cell-surface receptors or transfectincr agents, or by administerincr it in
:n t_1
linkacre to a horneobox- like peptide which is known to enter the nucleus (see e.g.,
Jollot et al., 1991, Proc. Nati. Acad.. . .

For antibodies, the dosacye administered to a patient is typically 0. I mg/kcr to
100 mcr/k(y of the patient's body weight. Preferably, the. . .
mcr/kcr to 10 mcy/ka of the patient's body weight. Generally, human 'bodies have a loncrer half-life within the human body than antibodies from other
anti L]
species due to the immune response to the foreign polypeptides. Thus, lower dosages
C] Z.,
of human antibodies and less frequent administration is often possible. Further, the
dosage and frequency of administration of antibodies of the invention may be reduced
rn

by enhanciner uptake and tissue penetration (e.g., into the brain) of the antibodies by
C tn
modifications such as, for example, lipidation.

Antibody-Based Diagnosis and Imaging
Labeled antibodies, and derivatives and analoas thereof, which specifically
L]
bind to a polypeptide of interest can be used for diagnostic purposes to detect,
zn
diaunose,. . . interest, comprising (a) assaying
the expression of the polypeptide of interest in cells or body fluid of an individual
usincFone or more antibodies specific to the polypeptide
interest and (b) comparing the
level of gene expression with a standard gene expression level, whereby an. . .
. .
assaying the expression of the polypeptide of interest in cells or body
n Zn
fluid of an individual using one or more antibodies specific
to the polypeptide interest
and (b) comparing the level of crene expression with a standard cyene expression level,
tn Z] L_
whereby. . . in the assayed polypeptide crene expression level
t,
compared to the standard expression level is indicative of a particular disorder. With
respect to cancer, the presence of a relatively hi oh amount
of transcript in blopsied
L]
tissue from an individual may indicate a predisposition for. . . may allow health
C y
professionals to employ preventative measures or aaaressive treatment earlier thereby
preventing the development or further progression of the cancer
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Antibodies of the invention can be used to assay protein
levels in a biological
sample using classical immunohistolocrical methods known to those. . .
see Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al.,
tn
J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based
methods useful for
detecting protein gene expression include immunoassays, such as the enz me linked
Zn C] y
immunosorbent assay (ELISA) and the radtojmmunoassay (RIA). Suitable antibody
assay labels are known in the art and include enzyme labels, such as, glucose oxidase;
C
radioisotopes, such as iodine ('1, 125i, 123i,. . .
. .
a human subject, the
In tn
quantity of radioactivity injected will normally range from about 5 to 20 millicuries of

LI
99mTc. The labeled antibody or antibody fragment
will then preferentially accumulate
at the location of cells which contain the specific protein. In vivo
tumor ima(yina is
described in S.W. Burchiel et al., Immunopharmacokinetics of
Radiolabeled
Antibodies and Their Fraoments. (Chapter 13 in Tumor
Imaging: The
Radiochernical Detection of Cancer, S.W. Burchiel and B. A.
Rhodes, eds., Masson
Publishing Inc. (1982).

Antibody-Based Kits
The present invention provides kits that can be used in the above
methods. In
one embodiment, a kit comprises an antibody of the invention,
preferably a purified
antibody, in one or more containers. In a specific embodiment,
the kits of the present
Invention contain a substantially Isolated polypeptide comprising an
epitope which i
C, I I is
specifically immunoreactive with an antibody included in the
kit. Preferably, the kits
of the present invention further comprise a control antibody
which does not react with
the polypeptide of interest. In another specific embodiment, the kits of
the present
invention contain a means for detectincr the binding of an
antibody to a polypeptide of
r_- tn
interest (e.g., the antibody may be conjugated to a
detectable substrate such as a
fluorescent compound, an enzymatic substrate, a radioactive compound or
a
luminescent compound., or a second antibody which recognizes
the first antibody may
be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a
diaonostic
kit for use in screening serum containincy antibodies specific
against proliferative
C] C LI
and/or cancerous polynucleotides and polypeptides. Such a kit
may include a control
antibody that does not react with the polypeptide of interest.
Such a kit may include a
substantially isolated polypeptide antigen comprising an epitope which
is specifically
immunoreactive with at least one anti-polypeptide anticren
antibody. Further, such a
kit includes means for detecting the bindina of said antibody
to the anticren (e.cr., the
antibody may be conjugated to a fluorescent compound
such as fluorescein or
rhodamine which can be detected by flow cytometry). In specific
embodiments, the
kit may. . .
. . .
kit
includes a solid support to which said polypeptide antigen is attached.
Such a kit may

also include a non-attached reporter-labeled anti-human antibody
. In this embodiment,
binding of the antibody to the polypeptide antigen can be
detected by binding of the
reporter-labeled antibody.

.
for use in
screenincy serum containina antiaens of the polypeptide of the
invention. The
t] Zn L_
diagnostic kit includes a substantially isolated antibody
specifically immunoreactive
with polypeptide or polynucleotide antigens, and means for detecting the
binding of
the polynucleotide or polypeptide antigen to the antibody. In
one embodiment, the
antibody is attached to a solid support. In a specific
embodiment, the antibody may be
a monoclonal antibody. The detecting means of the kit may
include a second, labeled
monoclonal antibody. Alternatively, or in addition, the
detecting means may include a
labeled, competing antigen.

After bindiner with specific antigen antibody to the reagent
and removing unbound
serum components by washing, the reagent is reacted with
reporter-labeled anti-
human antibody to bind reporter to the reauent in proportion
to the amount of bound
anti-antioen antibody on the solid support. The reagent is
again washed to remove
L] L] Zn
unbound labeled antibody, and the amount of reporter
associated with the reagent Is
determined. Typically, the reporter is an enzyme which is detected by.

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or kit for carryinGout this
Zn
diaunostic method. The kit Generally includes a support with surface-
bound
recombinant antigens, and a reporter-labeled anti-human antibody
for detecting
LI t)
surface-bound anti-anticren antibody.

LI
Therapeutic Compositions and Methods
The Tumor Necrosis Factor JNF) family ligands are known to be
among the most
pleiotropic cytokines, inducing a large number of cellular responses,
including cytotoxicity,
In C Z]
anti-viral activity, immunoregulatory activities, and the
transcriptional regulation of several
genes (D.V. Goeddel et al., Tumor Necrosis Factors: Gene
Structure and Biological
Activities, Symp. Quant. Biol. 51:597- 609 (1986), Cold Spring Harbor,
B. Beutler and A.

.
for studying tile phenotypic
effects that result from inhibiting TRAILFFRIO interactions on various
cell types. TRIO

polypeptides and antagonists (e. g. monoclonal antibodies to
TR IO) also may be used in in vitro
assays for detecting TRAIL or TRIO or the interactions thereof.

The therapeutic compositions and methods described in this section
include those
antibody-based composition and methods described in detail
above. For example, the agonists
and antagonists, and methods of using such acromsts and antagoists,
include the antibodies and
their uses described above.

. . .
treated or prevented by the polynucleotides, polypeptides and/or
acronists or antagonists
LI LI
of the invention include, but are not limited to, cancers
(such as f0111CL11ar lymphomas,
carcinomas with p53 mutations, and hormone-dependent tumors,
including, but not limited to
colon cancer, cardiac tumors, pancreatic
cancer, melanoma, retinoblastoma, alloblastorna, lung
cancer, intestinal cancer. testicular cancer
, stomach cancer, neuroblastoma, myxoma, myoma,
lymphoma, endothelloma, osteoblastoma, osteoclastorna, osteosarcoma,
chondrosarcoma,
adenoma, breast cancer, prostrate cancer, Kaposi's
sarcoma and ovarian cancer); autoimmune
disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's
thyrolditis, biliary
cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic
lupus erythernatosus and
immune-related glomerulonephritis. . . rejection. In preferred
embodiments, TRIO polynucleotides,
polypeptides, and/or antagonists of the invention are used to inhibit
growth, progression,
Z., zn
and/or metasis of cancers, in particular those listed above,
or in the paragraph that follows.

. . .
and chronic lymphocytic leukemia)), polycythemia vera, lymphomas
(e.g., Hodakin's disease and non-Hodgkin's disease), multiple myeloma,
Waldenstrom's
macroglobulinemia, heavy chain disease, and solid tumors
including, but not limited to,
sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma,
chondrosarcoma,
osteogenic sarcoma, chordoma, angiosarcoma, endotliellosarcoma,
lymphancriosarcoma,
lymphancyloendotheliosarcoma, synovioma, mesothelloma, Ewin(Y's
tumor, leiomyosarcoma,
rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast
cancer, ovarian cancer, prostate
cancer, squamous cell carcinoma, basal cell carcinoma,
adenocarcinoma, sweat gland
](0
carcinoma, sebaceous aland carcinoma, papillary carcinoma, papillary
adenocarcinomas,
cystadenocarcinoma. medullary carcinoma,. bronchocrenic carcinoma, renal
cell carcinoma,
hepatoma, bile duct carcinoma. chorlocarcinoma, serninorna, embryonal
carcinorna, Wilm's
tumor, cervical cancer, testicular tumor,
Juno carcinoma, small cell Juno carcinoma, bladder
carcinoma, epithelial carcinoma, aliorna, astrocytoma, medulloblastorna,

craniopharynatoma,
ependymoma, pinealoma, hemancrioblastoma, acoustic neuroma,
oligodendrogliorna,
Zn
menangioma, melanoma, neuroblastoma,. . .
. . .
invention
and agonists or anta(yonists thereof, are used to treat or prevent
autoimmune diseases and/or
inhibit the Growth, progression, and/or metastasis of cancers,
including, but not limited to,
tr C! tn
those cancers disclosed herein, such as, for example,
lymphocytic leukemias (Including, for
example, MLL and chronic lymphocytic leukemia (CLL)) and follicular
lymphomas. In
another. . . embodiment TR IO polynucleotides or polypeptides of the
invention and/or agonists or
antagonists thereof, are used to activate, differentiate or proliferate
cancerous cells or tissue
(e.o., B cell lineage related cancers (e.cr., CLL and MLL),
lymphocytic leukemia, or
lymphoma) and thereby render the cells more vulnerable to cancer
therapy (e. CF., chemotherapy
:n
or radiation therapy).
. . .
but
are not limited to, AIDS; neurodegenerative disorders (such as
Alzheimer's disease,
Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis
picrmentosa, Cerebellar
In
degeneration and brain tumor or prior associated disease);
autolmmune disorders (such as,
multiple sclerosis, S'ocyren's syndrome, Hashimoto's thyroiditis,
biliary cirrhosis, Behcet's
disease, Crohn's disease, polyrnyositis, systemic. . . myocardial
infarction,
stroke and reperfusion injury), liver injury (such as hepatitis related
liver injury,
ischemia/reperfusion injury, cholestosis (bile duct injury) and liver
cancer); toxin-induced liver
disease (such as that caused by alcohol), septic shock, cachexia and
anorexia. In preferred
embodiments, TRIO polynucleotides, polypeptides and/or agonists. . .
. . .
Fas include, but are not limited to, soluble Fas polypeptides;
Z__
mulitmeric forms of soluble Fas polypeptides (e.g., dimers of sFas/Fc);
anti-Fas antibodies
1-n
that bind Fas without transducina the biolocrical sianal that results in
apoptosis; anti-Fas-licrand
Z_ I= tr
antibodies that block binding of Fas-licrand to Fas; and
muteins of Fas-licrand that bind Fas but
C] U_
do not transduce the biological signal that results in apoptosis.
Preferably, the antibodies
In
employed according to this method are monoclonal antibodies.
Examples of suitable agents for
L_ zn

blocking Fas-ligand/Fas interactions, including blocking anti-Fas monoclonal antibodies, are
C LI zn
described in International application publication number WO 95/10540, hereby incorporated
by reference.

. . .

receptor
'bodies that bind the TRAIL receptor without transducing the biolocrical signal that results in
anti I I I zn tn
apoptosis, anti-TRAIL antibodies that block bindina of TRAIL to one or more TRAIL
receptors, and muteins of TRAIL that bind TRAIL receptors but do not transduce the biological
signal that results in apoptosis. Preferably, the antibodies employed according to this method
in
are monoclonal antibodies.

. . .

ancyiocyenesis is strincrently regulated and spatially and temporally delimited. Under conditions of pathological anglogenesis such as that characterizing
tr :n Z-- LI
solid tumor crrowth, these regulatory controls fall. Unregulated ancriocrenesis becomes
C LI LI tn Z71
pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number
of serious diseases are dominated by abnormal neovascularization including solid tumor errowth
and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by
Moses et al., Biotech. 9:630-634 (199 I)-, Folkman et al., N. Engl. J. Mcd., 333:1757-1763
(1995); Auerbach et al., J. Microvasc. Res. 29:401-411 (1985); Folkman, Advances in Cancer
Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz,
Am. J. Opthahnol. 94:715-743 (1982); and Folkman et al.,. . .

4n zn
For example, significant data have accumulated which suggest that the growth of solid tumors
LI zn
is dependent on ancylocrenests. Folkman and Klagsbrun, Science 235:442-447 (1987).

. . .

L]
which can be treated with the polynucleotides and polypeptides of the invention include, but are
not limited to those malianancles, solid tumors, and cancers described herein and otherwise
known in the art (for a review of such disorders, see Fishman et al., Medicine, 2d Ed.,. . .

. . .

glaucoma, diabetic
retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular
degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases,
ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.cr
reviews by Waltman et al., Am. J. Ophthal. 85:704-710 (1978) and. . .

of
blood composition-affecting disorders, such as, for example, hemophilia, cystic fibrosis,
pregnancy, menstrual disorders, early anemia of prematurity, spinal cord injury, aGing,
In
various neoplastic disease states, and the like. Examples of patient conditions that require
supplementation of the oxygen carrying capacity of blood and which.

dog, cat, non-human primate, and human, most
preferably human) to boost the immune system to produce increased quantities of one or more
antibodies (e.pr., IcrG, I(YA, IcYM, and I(YE), to induce
higher affinity antibody production (e.cr
L] t__ t_ Z:] Z-, L_
IgG, IcrA, IcrM, and IcrE), and/or to increase an immune response.

to an animal (Including, but not limited to, those listed above, and also
includincy transcrenic animals) incapable of producing functional endogenous antibody
t] Z-- tn
molecules or having an otherwise compromised endocFenous immune system, but which is
zn
capable of producing human immunocriobulin molecules by means.

An adjuvant to enhance tumor-specific immune responses.

As an auent to induce higher affinity antibodies.

As an auent to boost immunoresponsiveness amoncr B cell 'mmunodeficient individuals. B cell immunodeficiencies that may be ameliorated or
treated by. . . or polynticleotides of the invention, or agoilists thereof, include, but are not
limited to, SCID, conuenital a(yammaolobulinemia, common variable immunodeficiency,
Wiskott-Aldrich Syndrome, X-linked immunodeficiency with hyper ICYM, and severe combined
immunodeficiency.

in vitro or in vivo. Moreover, in
related embodiments, said enhancement or antagonization of anti cren presentation may be useful
as an anti-tumor treatment or to modulate the immune system.

As a means to induce tumor proliferation and thus make it more susceptible to anti-
neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus
Zn Z71
refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more
r]
their susceptibility profile would likely change.

As an anticFen for the aeneration of antibodies to inhibit or enhance TRIO mediated
responses.

Antacronists of TRIO include binding and/or inhibitory

antibodies, antisense nucleic
ribozymes or soluble forms of the TRIO receptor(s) (e.a,. the TRIO-Fc
molecule
aci
described in Example 38). These would be expected. . .
. . .
treatment or prevention of a wide rancre of diseases
and/or conditions. Such diseases and conditions include, but are not
limited to, cancer (e.g.,
immune cell related cancers, breast cancer, prostate
cancer, ovarian cancer, follicular
lymphoma, cancer associated with mutation or alteration of
p53, brain tumor, bladder cancer,
uterocervical cancer, colon cancer, colorectal
cancer, non-small cell carcinoma of the luna
small cell carcinoma of the luna, stomach cancer, etc.),
lymphoproliferative disorders (e.(Y
t, t]-q
lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection
(e.g., HIV-1 infection,
r_1 LI
HIV-2 infection, herpesvirus infection (including, but. . .
. . .
decreased apoptosis or decreased cytokine and adhesion molecule
expression
is exhibited. An agonist can include soluble forms of TRIO and
monoclonal antibodies directed
against the TRIO polypeptide.
. . .
I ]] I I in
increased apoptosis or NFkB expression is exhibited. An antacronist can
include soluble forms
of TRIO and monoclonal antibodies directed a(yainst the TRIO
poiypeptide.

:n
Another screening technique well known in the art involves expressing in
cells a
]n
construct wherein the receptor is linked to a phospholipase C
or D. Exemplary cells include
endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The
screening may. . .
. . .
as, for example, TNF family figand peptide fragments, transforming
growth
L- LI Z] Z]
factor, neurotransmitters (such as crlutamate, dopamine, N-
methyl-D-aspartate), tumor
tn
suppressors (p53), cytolytic T cells and antimetabolites. Preferred
agonists include
Z!,
chemotherapeutic drugs such as, for example, cisplatin,
doxorubicin, bleomycin, cytosine
L]
arabinoside, nitrocren mustard, methotrexate and vincristine. Others
include ethanol and -
1-n
arnyloid peptide. (Science 267:1457-1458 (1995)). Further preferred
agonists include
polyclonal and monoclonal antibodies raised acrainst the TRIO
polypeptide, or a fragment
C :n

thereof. Such acronist antibodies raised acyalnst a TNF-family receptor are disclosed in L.A.

. . .

Cowpox virus
crinA, Epstein-Barr virus BHRF], LMP-1, African swine fever Virus LMVV5-HL, and
Herpesvirus yl 34.5). calpain inhibitors, cysteine protease inhibitors, and tumor promoters
(such as PMA, Phenobarbital, and 0-Hexachlorocyclohexane).

. . .

L_ L_ r_- In
(1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5:539-549 (1988)). To this end,
the oligonucleotide may be conjugated to another molecule,
e.g., a peptide, hybridization
Z] Zn
triggered cross-linkiner auent, transport acrent,
hybridization-trl(ycrered cleavacre acrent, etc.

. . .

the cell surface bound forms of the receptor for
L_ In
bindincy to TNF-family ligands. Antagonists of the present invention also include antibodies
t]'
specific for TNF-family licrands and TRIO-Fc fusion proteins.

Montgomery et al., Eur. Cytokine Newt. 7:159 (1996). Further, antibodies specific for the
extracellular domain of this block HSV-I entry into cells. Thus, TRIO antagonists of the
present invention include both TRIO amino acid sequences and antibodies capable of
preventing mediated viral entry into cells. Such sequences and antibodies can function by
either competing with cell surface localized for binding to virus or by directly blocking binding
In
of virus to cell. . .

Antibodies accordincr to the present Invention may be prepared by any of a variety of
methods usino TRIO immunogens of the present. . .

Polyclonal and monoclonal antibody aconists or antagonists accordiner to the present
Zn C] Zn
invention can be raised according to the methods disclosed herein and and/or. . .

. . .

addition, due to lymphoblast expression of TRIO, soluble TRIO agonist or zn
antagonist mABs may be used to treat this form of cancer.

. . .

of the
invention (see generally, Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in
t:] tn
the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New
York, pp. 317 -327 and 353-365 (1989)). Liposomes containing TRIO polypeptide my be
prepared by methods. . .

. . .

Chemotherapeutic agents that may be administered with the

t] zn
compositions of the invention include, but are not limited to, antibiotic derivatives (e.g.,
doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrocrens (e.cr., tamoxifen);
antimetabolites (e.cr., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b,
tr
glutamic acid, plicamycin, mercaptopurme, and 6-thloguanine); cytotoxic. . .

In a specific embodiment, compositions of the invention are administered in
combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or
any combination of the components of CHOP. In another embodiment, compositions of the
invention are administered in combination. . .

. . .
Welch Medical Library. The relationship between Genes
In
and diseases that have been mapped to the same chromosomal region are then identified
through linkage analysis (coinheritance of physically adjacent genes).

. . .
wa
Z__ Zn y
io as to produce that polypeptide with the six His residues (i.e., a 6 X His tacy) covalently linked
In
to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding
sequence is inserted such that translation of the. . .

. . .
a
polyadenylation si anal arranged so that a cDNA conveniently can be placed under expression
Z--
control of the CNIV promoter and operably linked to the SV40 intron and the polyadenylation
signal by means of restriction sites in the polylinker.

. . .
37:767 (1984). The fusion of
the HA tacr to the target protein allows easy detection of the recombinant protein with an
t] Zn
antibody that recognizes the HA epitope.

. . .
Expression of the TRIO-FLA fusion protein is detected by radiolabelling and
immunoprecipitation, using methods described in, for example Harlow et al., Antibodies: a
Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
L] In
(1988). To this end. two days after. . . Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-
specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE crels
and autoradlography. An expression product of the expected size is seen in. . .

. . .

C] L_
the target enzyme, DHFR, as a result of amplification of the DHFR crene. If a second gene is
tn t] t)
linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art
In
that this approach may be. . .
. . .
expressed soluble His-FLAG-tagured TRAIL. The resulting complex was
In Z__
precipitated with protein G-Sepharose and bound TRAIL detected by Western blotting with
anti-FLAG antibody. Like DR4, DR5, and TR5 (TRID), TRIO bound TRAIL. Corroborating
this ability to bind TRAIL was the finding that TRIO-Fc, like. . .
. . .
is capable of
-5 substantially attenuating TRAIL-induced cell death, sucy(yestincr that TRIO antacronizes TRAIL
Z7] C11 In L_
siernaling
L_ tn'
Example 6
Production of an Antibody
a) Hybridoma Technology
The antibodies of the present invention can be prepared by a variety of
methods. (See, Current Protocols, Chapter 2.) As one example of such methods,
cells expressing TRIO are administered to an animal to induce the production of sera
containing polyclonal antibodies. In a preferred method, a preparation of TRIO protein
is prepared and purified to render it substantially free of natural contaminants.. . .

Monoclonal antibodies specific for TRIO protein are prepared using hybridoma
rn
technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol.

Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681
(1981)). In general, an animal (preferably a mouse) is immunized with TRIO
polypeptide or, more. . .
. . .
et al. (Gastroenterology 80:225-232 (198 1). The hybridoma cells obtained through such a selection are then assayed to identify clones which
secrete antibodies capable of binding the TRIO polypeptide.

Alternatively, additional antibodies capable ofbinding to TRIO polypeptide can
be produced in a two-step procedure using anti-idlotypic antibodies. Such a method
makes use of the fact that antibodies are themselves antioens, and therefore, it is
C
to obtain an antibody which binds to a second antibody
. In accordance with
possi 1

this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the TRIO protein-specific antibody call be blocked by TRIO.

Such antibodies comprise anti-ldlotypic antibodies to the TRIO protein-specific 'body and are used to immunize an animal to induce formation of further TRIO anti I protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is humanized. Such 'bodies can be produced using crenetic constructs derived from hybridoma cells anti I :__ t] producing the monoclonal antibodies described above. Methods for producing Z] tn chimeric and humanized antibodies are known in the art and are discussed infra. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BloTechniques 4:214 (1986);. . . al., WO 8601533; Robinson et al., WO 8702671; Boullanne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Acrainst TRIO From A Library Of scFvs

L]

Naturally occurring V-crenes isolated from human PBLs are constructed into a 1-In I,, library of antibody fragments which contain reactivities a(Yainst TR IO to which the In donor may or may not have been exposed (see e.g., U.S.. . . . . library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phacre displaying antibody fragments, approximately 109 E. coil harboring the phacremid are Zn r_1 Z__ used to inoculate 50 ml of 2xTY containing 1% glucose. . . . . for precise mapping are t] Z1_ L] obtained usincr a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination L] Zn with a cooled charcre-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection,. . .

For example, antibody-sandwich ELISAs are used to detect TRIO in a sample, preferably a biological sample. Wells of a microtiter plate are coated

with specific antibodi
]] I I I I I ies to
TRIO, at a final concentration of 0.2 to 10 ucy/ml. The
antibodies are either monoclonal or
LI
polyclonal and are produced using technique known in the art. The wells
are blocked so that
r_l
binding of. . .

Next, 50 ul of specific antibody-alkaline phosphatase
conjugate, at a concentration of
I 0 25-400 ncr, is added and incubated for 2 hours at room temperature.
The plates are again
washed three times with delonized or distilled water to remove unbounded
conjugate.

Preferred antagonists for use in the present invention are TRIO-specific
antibodies.

Antisense technology is used to inhibit production of TRIO. This
technology is one
In n
example of a method of decreasing levels of TRIO polypeptide, preferably
a soluble and/or
secreted form, due to a variety of etiologies, such as cancer.
. . .
RNA) TRIO sequences into an animal
to increase or decrease the expression of the TRIO polypeptide. The TRIO
polynucleotide may
be operatively linked to a promoter or any other (Yenetic
elements necessary for the expression
of the TRIO polypeptide by the taraet tissue. Such. . .
. . .
flanking the promoter. The tarcreting sequence will be
Zn Zn
sufficiently near the 5'end of TRIO so the promoter will be operably
linked to the endoCFenous
Zn
sequence upon homologous recombination. The promoter and the targeting
sequences can be
r, r_l Zn
amplified usiner PCR. Preferably, the. . .

Once the cells are transfected, homologous recombination will take place
which results
C)
in the promoter beiner operably linked to the endocrenous TRIO
sequence. This results in the
expression of TRIO in the cell. Expression may be detected by
immunological. . .
. . .
and 88% N,) tissue culture incubator, and after 7 days, analyzed for
expression of
differentiation antigens by staining with various monoclonal
antibodies and FACScan.
. . .
88% N 2)
L] rn
tissue culture incubator, and after 7 days, analyzed for expression of
differentiation antigens by
staining with various monoclonal antibodies and FACScan.
. . .
using a necrative selection procedure, where the committed cells of

most of the lineages are removed using a panel of monoclonal antibodies (anti cd I I b, CD4.

. . .

which purified tonsillar B cells
are cultured in the presence of either formalin-fixed Staphylococcus aureits Cowan I (SAC) or
immobilized anti-human I(YM antibody as the priming acrent.
Second signals such as IL-2 and
C-1 in Zn
IL-15 synercrize with SAC and IgM crosslinking to elicit. . .

. . .

Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also
used as a control which enhances proliferation. Control antibody which does not induce
proliferation of T cells is used as the necrative controls for the effects of TRIO proteins.

. . .

PBS containing
1 % BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate
FITC- or PE-labeled monoclonal antibodies for 30 minutes at
4'C. After an additional wash,
the labeled cells are analyzed by flow cytometry on a FACScan (Becton.
. .

. . .

PBS containino I% BSA and 0.02 rnM sodium azide, and then incubated with 1:20
C
dilution of appropriate FITC- or PE-labeled monoclonal
antibodies for 30 minutes at 4'C.

. . .

Vascular Endothelial Cells
For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-
dimethylthiazol yl) (3-carboxymethoxyphenyl) (4-sulfophenyl)2H-tetrazollum) assay
with the electron coupling reagent PMS (phenazine
methosulfate) was performed (CellTiter 96
AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1. . .

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-
human keratin antibody using ABC Elite detection system. Human skin is used as a positive
tissue control while non-immune IgG is used as a. . .

Proliferating cell nuclear anticyen/cyclin (PCNA) in skin specimens is demonstrated by
In
using anti-PCNA antibody (1:50) with an ABC Elite detection
system. Human colon cancer
served as a positive tissue control and human brain tissue is used as a negative tissue control.

Each specimen included a section with omission of the primary antibody and substitution with
non-immune mouse IcFG. Ranking of these sections is based on the extent of proliferation on a
scale of 0-8,. . .

. . .

was prepared that consists of a soluble form of TRIO
(corresponding to amino acids -55 to 149 of SEQ ID NO:2) linked

to the Fc portion of a human
-5 IcrG I immuno(yloulin molecule. The ability of this protein to alter the proliferative. . .
. . .
as priming acrent and
In L- 1-In
Neutrokine-alpha as a second signal (data not shown). It is important to note that other Tumor
Necrosis Factor Receptors (TNFR) fusion proteins (e.g., DR4-Fc (Internatioaril Application
Publication No. WO 98/32856), TR6-Fc (Internatioaril Application Publication No. WO
98/31799), and TR9-Fc. . .
. . .
ID NO:2; and a polypeptide
comprising amino acid residues from about 195 to about 228 in SEQ ID NO:2
tn
22. An isolated antibody that binds specifically to a TRIO receptor polypeptide of

CLMEN 28 The antibody of claim 22 that is an scFv fragment.
LI

29 The antibody of claim 22 that is an Fab fragment.
rn
Figure IA
10 30 50
CCACCC-%C:GCGTCCCCCCACGCGTCCGGAGA,-A,CCTTTCvC-'%CCCGC.XCA]2%ACTACGGGC-AC
70 90 110
GAT=cTCATTGATTTTTGGCGC=CGATCC-A,CCCTCCTCCCTTCTC.NTC-GGACTTTGG
130 150 170
GC-ACA,AAGCGTCCCCACCGCCTCGAGCGCTCGACCAGGC-CGCT-A.TCC.A.GGACCCAGGA.C-k
G P T A S -. . . S? T -1 z A C - - - ttjoL#v A T I I 5 I ali a IL =3
P75
NA i 7PIJL-R] a rmml 3 0 HWAi 3 L Z LQ 9G &C L.IMQ E SHM T 111RAZZ L C.
. . ng/ml SCF
c
o0aft
c O] + 1 ng/ml IL-3 and
E 5,000
5 n9iml SCF
2,500
low
.0 Le
La
TRI 0 (ng/ml)
SEQUENCE LISTING
<110> Human Genome Sciences, Inc.
<120> Human Tumor Necrosis Factor Receptor TR10
<130> PF379PCT2
<140> Unassigned
<141> 2000 25
<150> 60/136,786
<151> 1999 28
<150> 60/142,563
<151> 1999 07
<150> 60/144fO23
<151> 1999 ILS
<160> 16
<170> PatentIn Ver.. . .